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CONTENTS

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ERRATA AND AUTHORS' EMENDATIONS

Page 3, line 16, add "25° C."

Page 9, line 2, "either sucrose or reducing sugars" should be "sucrose content."

Page 76, table 3, Soil no. 312, Mar. 2, Eh "+.030" should be "−.030."

Page 102, table 4, last line of heading, "July balance" should be "Joly balance."

Page 110, table 6, in third line of heading, "0" should be "0.5."

Page 153, line 7, "earliest culm" should be "tallest culm."

Page 155, sixth line from bottom, "greater portion" should be "a large number."

Page 189, table 2, the first brace is incorrectly shortened by one number, thereby making all subsequent brace inclusions wrong except the last.

Page 229, table 2. The following note should be inserted immediately above the footnotes: "In 1932, normal cows 2, 3, 4, and 6 gave negative reactions; in 1933, udderless-control cow 35 and normal-control cows 6 and 7 were negative; in 1934, udderless-control cow 35 and normal-control 4 were negative."

Page 314, line 7, under heading "Discussion", "(1, 19, 23)" should be "(2, 19, 23)."

Page 314, twelfth line from bottom, "(6, 7, 29)" should be "(7, 29)."

Page 411, table 8, under Family 81.22, last column (difference) should read "2.0, 1.4, 2.3, .1, −.3, .4, −.2, − 2, −.5, .4, 0, −.4, .1, 0, −.7, and .1."

Page 419, line 5, "raw-cooked" should be "raw, cooked."

Page 419, footnote, reference number 4 to literature should be italic and enclosed in parenthesis.

Page 422, table 3, last line of heading, comma should be inserted after "*series 2.*"

Page 453, paragraph 3, line 5, "15 to 40" should be "15 to 25."

Page 453, fifth line from bottom, "typical" should be "special."

Page 458, table 3, second column from right under "First year", Desiccated meat meal, "5." should be "5.3."

Page 504, line 5, under "Backcross Data", "white" should be "yellow."

Page 516, figure 1, cells labeled "A" are described in legend "C" and vice versa.

Page 643, legend under plate 6 should appear under plate 7 and legend under plate 7 should appear under plate 6.

Page 666, table 6, omit "Infected" in the two box heads.

Page 668, seventeenth line from bottom, "table 6" should read "table 7."

Page 675, table 1, last column, third number should be "15."

Page 752, figure 1. This illustration is upside down.

Page 833, the formula at the bottom of the page should be "Concentration (grams per 100 cc extract) $=35.51-\sqrt{1261-9.615\ (OR_a - OR_b)}$."

Page 841, lower right-hand corner, key no. should be "K-279."

Page 845, table 1, column 5, last line under Phenol, " 5 1,420" should read "> 5 1,420."

Page 862, table 1, "cc units" should follow "Alkalinity of ash" and be deleted after "Vitamin A" and "Vitamin C."

Page 876, table 4, columns 4, 6, 8, and 10, "Ratio to total dry matter" should be "On basis of total dry matter." Columns 5 and 7, "Total dry matter insoluble in 70-percent alcohol" should be "On basis of dry matter insoluble in 70-percent alcohol." Columns 9 and 11, "Total dry matter soluble in 70-percent alcohol" should be "On basis of dry matter soluble in 70-percent alcohol."

Page 944, table 4, first line of heading, "loteners" should be "lateness."

EFFECT OF CARBON DIOXIDE ON THE CARBOHYDRATES AND ACIDITY OF FRUITS AND VEGETABLES IN STORAGE [1]

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INTRODUCTION

The effect of carbon dioxide storage on carbohydrate transformation in certain fruits and vegetables was discussed in an earlier paper (*18*).[3] The present paper supplements the previous one by presenting data on three additional points: (1) Carbohydrate transformation in vegetables following removal from carbon dioxide storage, (2) carbon dioxide storage of several products not previously included, and (3) the effect of carbon dioxide storage on the acidity of certain fruits and vegetables.

The first point was suggested by inquiries regarding the earlier work. The question was raised as to how long treated peas and corn would maintain a high sugar content after removal from carbon dioxide storage and to what extent this advantage could be passed along to the ultimate consumer. The acidity studies were suggested by flavor tests conducted on fruits following removal from carbon dioxide storage. Several individuals found that there was a loss in sourness or astringency in the treated fruits.

REVIEW OF LITERATURE

The rapid deterioration in quality of freshly harvested fruits and vegetables has led to a study of the chemical changes that take place in these products and to an attempt to store them under conditions best adapted to maintenance of quality. Carolus (*7*) was able to retard the loss of total sugar in shelled green (Henderson Bush) lima beans by storing them at 0° to 2° C. Where the beans were held in cellophane bags, much of the total sugar was retained but the flavor was usually impaired. The sugar loss was retarded to some extent by holding the beans unshelled. Leaving them on the vine was more effective than the cold-storage treatment.

Several writers have shown that the quality of carrots deteriorates in storage. Hasselbring (*14*) found that the principal changes were a conversion of sucrose to reducing sugars and a transformation of polysaccharides to simple sugars. These processes occurred more rapidly at the higher temperatures, and under constant conditions tended to reach equilibrium after about 10 weeks. After this time there was a slight increase in sucrose. Platenius (*21*) reported similarly that sucrose in stored carrots was converted to reducing sugars at first but that the process was later reversed.

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The possible effect of carbon dioxide on acidity was suggested as early as 1920, when Sando (*22*) noted that tomatoes, picked green and ripened without ventilation, showed high titratable acidity. He suggested that incomplete oxidation of carbohydrates to carbon dioxide was responsible for the accumulation of the acid.

In 1924 Magness and Diehl (*17*) treated Winesap and Delicious apples with various percentages of carbon dioxide for 10 days at 22° C. The total acidity disappeared more rapidly in the higher concentrations of carbon dioxide, the results being less pronounced for Delicious than for Winesap varieties.

Thornton (*23*) reported on carbon dioxide treatment of plants and various types of plant tissues, such as fruits, roots, stems, tubers, and bulbs, at 2°, 5°, 15°, and 25° C. Carbon dioxide in the presence of oxygen (30–60 percent CO_2 and 20 percent O_2) resulted in a decrease in hydrogen-ion concentration of the sap extracted from the tissue. Maximum differences were obtained at 25° during the period between 96 and 168 hours of storage. The reduction in hydrogen-ion concentration was found to be dependent upon the presence of oxygen in the gas mixture, and where this gas was lacking there was an increase in hydrogen-ion concentration.

Kidd and West (*16*), working with Lane's Prince Albert apples, report that increasing the concentration of carbon dioxide accelerated the loss of acid, but that reducing the concentrations of oxygen had little effect on acid loss. The concentrations of carbon dioxide employed were 5, 10, and 15 percent.

Hartman and Bullis (*12*) report no striking chemical or physical changes in stored cherries except losses of weight and volume, and state that there may be even a slight increase in the acidity of the juice if the cherries are held long enough. Nightingale, Addoms, and Blake (*19*) report an increase in acidity of Elberta and Shipper Cling peaches with development until the fruit reaches the soft ripe stage, when the percentage of acid suddenly decreases. Allen (*1*) found that, generally speaking, there is little significant change in acid content of peaches with maturity. A slight decrease continues after harvest, but this does not reach its minimum until the fruit has passed its prime eating condition.

The effect of low temperatures on flavor and subsequent ripening of mature green tomatoes has been discussed by a number of investigators. Diehl (*8*) stated that the exposure of mature green Globe tomatoes to a temperature just above the freezing point was not harmful if the exposure did not extend beyond 5 days. He also found that if mature green Acme, Stone, and Globe tomatoes were held at 32° F. for 8 days and then removed to a warm room they broke down and decayed without losing the green color.

Wardlaw and McGuire (*24*) were able to hold mature green tropically grown tomatoes at 47.5° F. for 20 days and then ripen them at 70°. Wright et al. (*25*) stored mature green tomatoes at 32°, 36°, 40°, and 50° for various periods and then transferred them to 60° to ripen. They found that the fruit ripened but failed to color properly if the preliminary storage consisted of 8 to 11 days at 32° and 36°, 11 days or more at 40°, or more than 14 days at 50°. Barker (*4*) found that the storage of tomatoes below 50° resulted in physiological injury. Haber (*11*) reported similar results. Sando (*22*)

reported that tomatoes picked green and ripened without ventilation were inferior in flavor and that coloring was retarded. Kidd and West (15) reported that carbon dioxide of higher concentration than 5 percent at 12° C. had an injurious effect on tomatoes.

MATERIALS AND METHODS

The peas, beans, corn, carrots, and plums used in these experiments were grown at Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. The peaches were obtained at Leesburg, Va., and transported to Washington by motor truck. Tomatoes and cherries were purchased from the local wholesale market. All materials were brought to the laboratory early in the morning and placed under experimental conditions as rapidly as possible. As a rule the experiment was started within an hour after the arrival of the fruit at the laboratory.

The experimental material was placed in 5-gallon glass pickle jars and held at 0°, 5°, 10°, 15°, and 20° C. Constant temperatures and constant gas percentages were maintained as previously described (5). In the latter half of these experiments a slight departure was made from the former method of mixing carbon dioxide with air. The air and the carbon dioxide were supplied directly to the mixing chamber by cylinders of compressed gases, the desired percentage and volume being obtained by means of a reducing valve and flow meters. An additional cylinder of compressed air was connected to the control lots and adjusted to secure a constant renewal of the storage atmosphere comparable to that of the treated lots.

If solid carbon dioxide is used as a supplementary refrigerant for fruits and vegetables the gaseous carbon dioxide may run as high as 40 to 50 percent during the first 24 hours, and it was the object of this investigation to study the biochemical effects of such a percentage of carbon dioxide on the product.

Large quantities of the fruits and vegetables were employed in order that they might be carefully culled and representative specimens selected for each lot. At the time of sampling the following quantities were removed from each lot: 500 g of peas, 500 g of lima beans, 500 g of cherries, 10 ears of sweet corn, 5 to 10 tomatoes, 10 peaches, 7 carrots, and 25 plums. These samples were thoroughly mixed, and 20 g and 25 g duplicates were preserved for carbohydrate determinations and 50 to 100 g for determinations of acidity.

The methods employed for sampling were essentially those prescribed for plants by the Association of Official Agricultural Chemists (3). Peas, beans, and corn kernels were dropped into sufficient hot 95-percent alcohol to make the final concentration of alcohol 80 percent. Peaches, tomatoes, plums, cherries, and carrots were minced in a food chopper before preserving in alcohol. The samples were boiled for 5 minutes and were then stored for subsequent analyses.

Official methods were employed for carbohydrate analyses. The fruits were extracted in a Soxhlet extraction apparatus with 80-percent alcohol until sugar-free. Peas, beans, corn, and carrots were dried after the preliminary extraction and ground to pass through a 40-mesh sieve before being transferred to the Soxhlet extractors.

Reducing sugars were determined by the Munson and Walker gravimetric method. Sucrose was determined as invert sugar after inversion with a commercial preparation of invertase that had been standardized with a Bureau of Standards sample of sucrose.

Moisture was determined by weighing 3 to 5 g of the mash, covering with absolute alcohol, and drying to constant weight in a vacuum oven at 65° C. and 15 inches of mercury.

Total acidity of the fruit juice was determined by straining the minced pulp through one thickness of cheesecloth and titrating aliquots with N/10 sodium hydroxide. Bromthymol blue was used as an indicator.

Carotene was determined by the method described by Palmer (*20, pp. 251–254*), with a Klett colorimeter for comparisons.

Hydrogen-ion determinations were made by means of a portable potentiometer, a quinhydrone electrode, and a calomel half-cell. The apparatus was standardized by means of a standard solution of acid potassium phthalate. Readings were also compared with those obtained with a bubbling hydrogen electrode, and it was found that the results recorded at the duration of 1 minute checked within 0.05 of a pH unit with the readings after equilibrium on the hydrogen electrode.

Total acidity and pH values on the total fruit pulp were obtained as follows: A 100-g sample of the minced material was covered with boiled distilled water and boiled for 1 hour. The mixture was made up to 500 ml when cooled to 20° C. This was filtered, and acidity and pH determinations were made on aliquots of the filtrate.

CARBON DIOXIDE AS A SUPPLEMENT TO REFRIGERATION

RATE OF COOLING OF LIMA BEANS AND SWEET CORN IN STORAGE

The importance of refrigerating green peas, lima beans, and sweet corn as soon as possible after harvest has frequently been stressed. One point, however, is rarely mentioned. There may be considerable lag in lowering the temperature of the vegetables to that of the storage room, both because of their high temperature at the time of harvest and because of the heat of respiration following harvest. Figures 1 and 2 illustrate this point. Henderson Bush lima beans (fig. 1) and Golden Giant sweet corn (fig. 2) were harvested in the forenoon, packed in bushel hampers, and placed in the 0° C. room at Arlington Farm at 1 p. m. Resistance thermometers were placed at various places in the pack, and the temperatures were read, without entering the room, by means of cables and a resistance box. As will be seen from the charts, the initial temperature of the vegetables was about 27°. During the first 2 hours the interior of the sweet-corn pack rose to 31.9°. After that the temperature began to drop. The temperature of the lima beans began to drop as soon as the beans were placed in storage. It is significant that it was nearly 20 hours before the temperature of the beans attained that of the storage room and 44 hours before the corn in the outer layers of the hamper attained this temperature. The center of the corn pack never attained the temperature of the storage room during the period of the observations.

IMPORTANCE OF EARLY TREATMENT WITH CARBON DIOXIDE

It is obvious, then, that since fresh vegetables under usual storage conditions are cooled down rather slowly, even when rushed into refrigeration as quickly as possible, the sugar content and freshness,
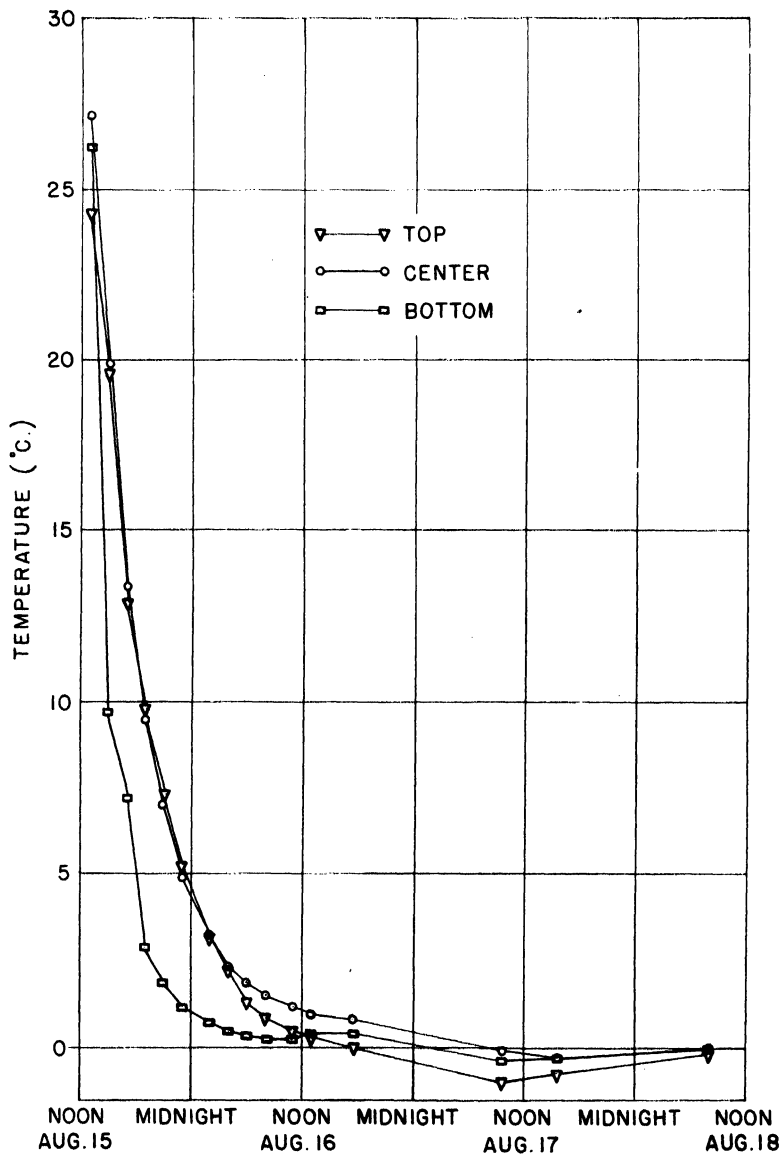
FIGURE 1.—Rate of cooling of Henderson Bush lima beans stored in bushel hampers at 0° C.

which are so desirable in certain vegetables, must be seriously diminished during the cooling process. For this reason it would seem advantageous to retard the respiration and sugar loss by producing an

atmosphere of carbon dioxide for the first 24 or 48 hours or until the pack is cooled to the desired temperature.
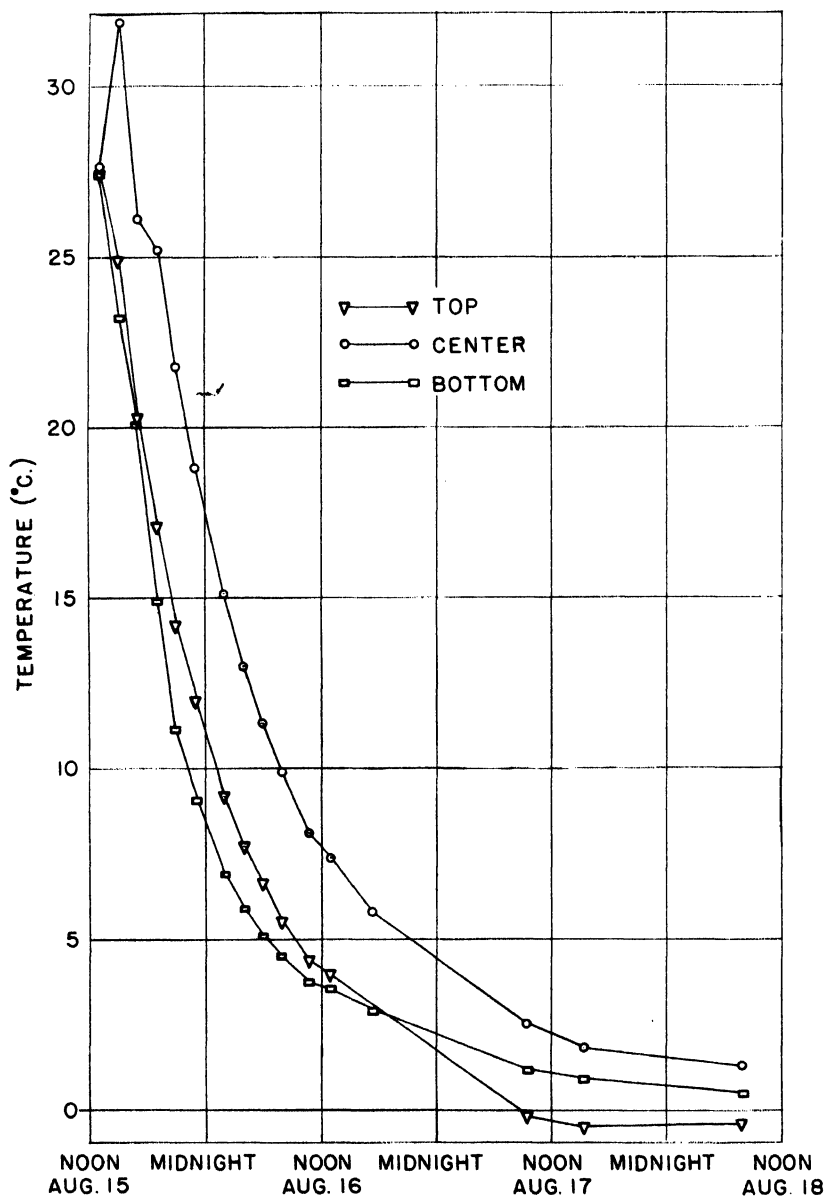
FIGURE 2.—Rate of cooling of Golden Giant sweet corn stored in bushel hampers at 0° C.

EFFECT OF CARBON DIOXIDE ON SWEET CORN CANNED IMMEDIATELY AFTER TREATMENT

As already mentioned, the sugar loss in sweet corn was retarded by carbon dioxide in storage experiments in the laboratory. In the summer of 1933 similar experiments were conducted at a canning

factory at Aberdeen, Md. Nine bushels of Country Gentleman (Shoe Peg) sweet corn selected at random from the supply reaching the factory early in the morning were mixed thoroughly and sorted into three equal lots. All three lots were stored immediately for 24 hours, as follows: Lot 1 was placed in a pony refrigerator [4] containing 10 pounds of solid carbon dioxide and 75 pounds of ice; lot 2 was placed in a pony refrigerator containing 100 pounds of ice; lot 3 was stacked on the floor of the canning shed. Gas determinations indicated that the carbon dioxide content of the atmosphere in the refrigerator containing lot 1 rose to 50 percent within 4 hours after the refrigerator was closed, and dropped to 37.6 percent when the refrigerator was opened after 24 hours' storage. Respiration in lot 2 raised the carbon dioxide content of the refrigerator atmosphere to 8.5 percent, 10 hours after the refrigerator was closed.

The outside air temperature at the beginning of the experiment was 23.3° C. The temperature of lot 1 had dropped to 10° by midnight, and that of lot 2 to 7.7°, while the center of the pile of lot 3 rose to 30°. At the time of opening, after 24 hours, the temperature of lot 1 was 6.9°, that of lot 2 was 6.1°, and that of the center of the pile of lot 3 was 27.7°.

The lots were removed from the refrigerators and from the stack on the floor, husked, cut from the ear, and canned immediately. Each lot was processed separately and the machinery was cleaned after each lot. In addition, a fourth lot of canned corn (lot 0) was reserved for flavor and sugar tests. This consisted of corn that had been canned immediately after arrival at the factory and was selected from straight factory run.

The canning process consisted in mixing a hot saline solution with the kernels, sealing, and processing for 40 minutes at 115.5° C. with 12 pounds' steam pressure.

All lots were taken to Washington, D. C., where they were tested for flavor and sugar content.

In order to subject the treated lots to as severe a test as possible, they were compared in flavor with that of the lot canned immediately (lot 0). Some of the persons testing the corn preferred the carbon dioxide treated lot, while others were able to detect something resembling an overcooked flavor in this lot. Rating all lots in the order of preference of the taster, and assigning a value of 100 to the lot that had been canned immediately, the others received the following values: Lot 1 (carbon dioxide), 72.8 ; lot 2 (ice), 70; lot 3 (stacked on floor), 47.1.

Fifty-gram samples were removed from cans of each lot, extracted with 80-percent alcohol, and analyzed for sugar according to official methods (*3*). Inasmuch as water had been added to the cans during the canning process, care was exerted to keep the contents well stirred while the sample was being taken. The variation in sucrose content of samples from duplicate cans in the same lot was never greater than 0.09 percent and averaged 0.05 percent. Comparing the total sugar in lots 1, 2, and 3 with that in lot 0 considered as 100, the percentages of sugar retained are as follows: Lot 1, 91.2; lot 2, 71.0; lot 3, 43.8.

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[4] A pony refrigerator is an insulated box employed for shipping perishable fruits, such as strawberries, from southern production areas to northern markets before carlot shipments are moving. It contains a shallow ice pan beneath which is a compartment having a capacity of 64 or 80 quarts.

Two weeks later the experiment was repeated. The second experiment was similar to the first in all details except that the corn was stored for 42 hours instead of 24 and most of it had attained the late milk or early dough stage of maturity as compared with the early milk stage of the corn used in the first experiment.

·In the flavor tests in this experiment a slight preference for lot 2 over lot 1 was shown. The ratings were as follows: Lot 1 (carbon dioxide), 72; lot 2 (ice), 74; lot 3 (stacked on floor), 53. Analyses for total sugar gave results similar to those in the previous experiment. Compared with lot 0 as 100, the percentages of sugar retained were as follows: Lot 1, 92.8; lot 2, 89; lot 3, 47.6.

IMMEDIATE EFFECT OF CARBON DIOXIDE STORAGE

CARBOHYDRATE TRANSFORMATION IN LIMA BEANS AND CARROTS

Henderson Bush lima beans stored in an atmosphere containing 42 percent carbon dioxide for 1 to 5 days reacted in a manner similar to that previously reported for peas (*18*). For the purpose of retaining the sucrose, a storage temperature of 5° C. was nearly as effective
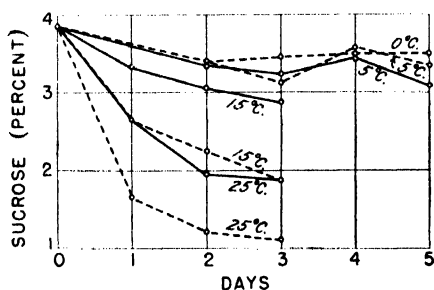
FIGURE 3. Sucrose content of Henderson Bush lima beans stored in an atmosphere containing 42 percent carbon dioxide for 1 to 5 days at various temperatures. Solid lines, treated; broken lines, control.

as 0°, and an atmosphere containing 42 percent of carbon dioxide made little difference at 5°. At 15° and 25°, however, the sugar loss was more rapid and the carbon dioxide was effective in retaining much of the sugar content (fig. 3). The lot stored at 25° with 42-percent carbon dioxide retained 69 percent of the original sugar on the first day and 51 percent on the second. The control lot at this temperature retained 43 and 31 percent on the first and second days, respectively. At 15° the carbon dioxide treated lot retained 86 percent of its original sugar on the first day and 79 percent on the second day. The control lot retained 68 percent of its sugar on the first day and 58 percent on the second. These differences in sugar content continued over the third day of treatment, but the carbon dioxide treated lots had lost their palatability on that day.

Figure 4 shows the results of the carbon dioxide studies on carrots. The carrots received 39-percent carbon dioxide for periods of 3 days. The values represent averages of two identical experiments performed at different times with similar lots of carrots. The sucrose content decreased gradually at 15° and 25° C., but the rate of loss was retarded in the lots treated with carbon dioxide. The greatest contrast was observed on the second day. At this time the lot treated with carbon dioxide at 25° had 1.5 times as much sucrose as the control, and the similar lot treated at 15° had 1.4 times as much.

The reducing sugars tended first to increase and then to decrease in both the treated and untreated lots. In all cases the percentage of reducing sugar was higher in the controls than in the lots treated

with carbon dioxide. At 5° C. there was little contrast between treated and control lots in either sucrose or reducing sugars.

Additional lots were held in carbon dioxide beyond the 3-day period to test the effect on flavor. The limit of tolerance appeared to be 9 days at 5° C., 6 days at 15°, and 4 days at 25°.

EFFECT ON ACIDITY OF FRUITS

At the time of the original studies of the effect of carbon dioxide upon carbohydrates in fruits, acidity studies were made on cherries and peaches stored for 24 and for 48 hours at 0°, 5°, 10°, 15°, and 20° C. Early Richmond cherries received 50-percent carbon dioxide for 24 hours. Elberta peaches were exposed to 40-percent carbon dioxide for 24 and 48 hours. The results appear in tables 1 and 2. The carbon dioxide exerted no consistent effect upon the acidity of the cherries or the peaches during the period of treatment.
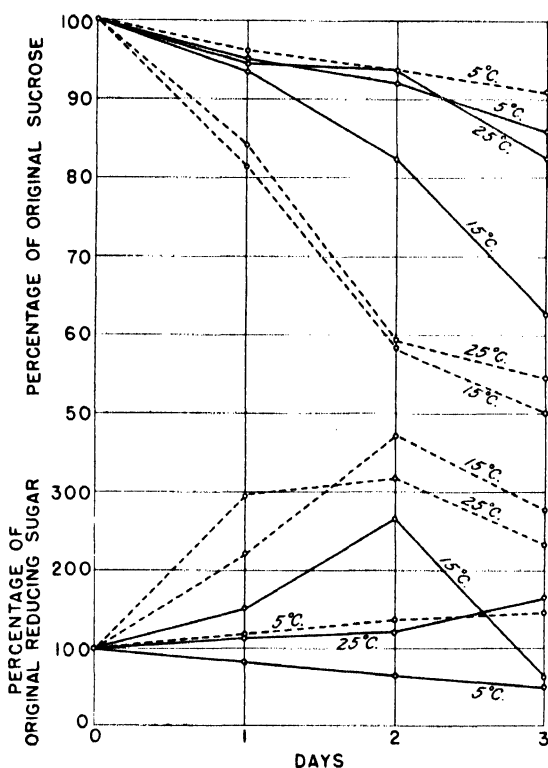
FIGURE 4. Sucrose and reducing sugar in Danvers Half Long carrots stored in an atmosphere containing 39 percent carbon dioxide for 3 days at various temperatures. Solid lines, treated; broken lines, control.

TABLE 1.—*Total acidity and pH of Early Richmond cherries after 24 hours' treatment with 50-percent carbon dioxide at various temperatures*

Temperature (° C.)	Acid in fresh pulp as malic		pH of fresh pulp		Temperature (° C.)	Acid in fresh pulp as malic		pH of fresh pulp	
	Treated	Control	Treated	Control		Treated	Control	Treated	Control
	Percent	*Percent*				*Percent*	*Percent*		
	[1] 1.37	[1] 1.37	[1] 3.47	[1] 3.47	10	1.50	1.45	3.41	3.40
0	1.45	1.42	3.45	3.50	15	1.47	1.33	3.44	3.45
5	1.44	1.47	3.43	3.40	20	1.33	1.47	3.46	3.44

[1] Original sample.

TABLE 2.— *Total acidity of Elberta peaches treated with 40-percent carbon dioxide for 1 and 2 days at various temperatures*

Temperature (° C.)	Days	Acid in pulp as malic		Temperature (° C.)	Days	Acid in pulp as malic	
		Treated	Control			Treated	Control
		Percent	*Percent*			*Percent*	*Percent*
		[1] 0. 74	[1] 0. 74	5	1	0. 68	0. 66
25	1	. 59	. 68	5	2	. 66	. 72
25	2	. 71	. 71	0	1		. 70
15	1	. 67	. 66	0	2		. 64
15	2	. 66	. 73				

[1] Original sample.

Marglobe and Earliana tomatoes were treated for 6 days with 50-percent carbon dioxide at temperatures of 0°, 10°, and 20° C. The results appear in figures 5 and 6. In general there was a gradual decrease in acid and hydrogen-ion concentration at the higher temperatures during storage, but the decrease was greater in the carbon dioxide treated lots. This reduction in acidity, due to the gas treatment, was more pronounced for the hydrogen-ion concentration than for the total acidity. It will be noted, for instance, that the hydrogen-ion concentration was lower in the treated lots than in the controls at both 20° and 10°,

FIGURE 5.—Hydrogen-ion concentration of fruit pulp and fruit juice of Earliana tomatoes and fruit juice of Marglobe tomatoes treated with 50-percent carbon dioxide for 6 days at 0°, 10°, and 20° C. Shaded bars, carbon dioxide treated; plain bars, control.

whereas the total acidity was lower in the treated lots at 20° only. There was practically no reduction in acidity in either the treated or untreated lots stored at 0°.

Burbank plums treated with 50-percent carbon dioxide for 6 days at 0°, 10°, and 20° C. responded in a manner somewhat similar to that of tomatoes in regard to acidity changes. Figure 7 shows the percentage of titratable acidity in the juice after 3 and 6 days' treatment with 50-percent carbon dioxide. The results of two experiments have been averaged. The hydrogen-ion concentration showed a slight tendency to decrease with carbon dioxide treatment, but the results were not very consistent and were not considered significant. Differences in the total acidity are not great, but as a rule the carbon dioxide treatment seems to have reduced the acidity, especially at

temperatures of 10° and 20°. Because of too great a loss from decay, the 20° controls were not sampled on the sixth day. The lots treated with carbon dioxide at 20° were all sound. There was a slight rise in acidity between the third and the sixth day, but the treated lots were still lower in acid than the controls.

The Burbank plums were analyzed for benzoic acid content before and after treatment with carbon dioxide, but no significant differences were found.

GLYCOSIDES [5] OF PEACHES AND CARROTS

Peaches and carrots treated with carbon dioxide were analyzed for glycosides by Harvey's method (13) for phloridzin. Significant quantities of these substances were not obtained by the method employed.
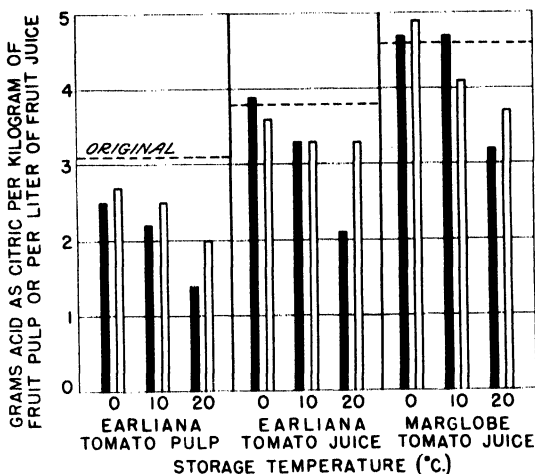
FIGURE 6. Total acidity of fruit pulp and fruit juice of Earliana tomatoes and fruit juice of Marglobe tomatoes treated with 50-percent carbon dioxide for 6 days at 0°, 10°, and 20° C. Calculated on the basis of grams acid as citric per kilogram of fruit pulp or per liter of fruit juice. Shaded bars, carbon dioxide treated; plain bars, control.
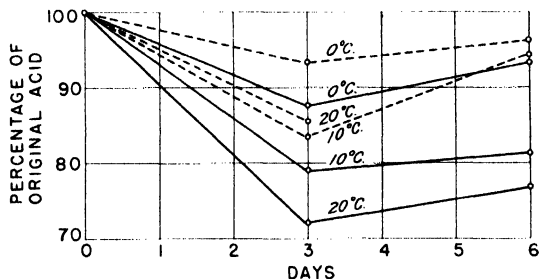
FIGURE 7.—Percentage of original titratable acidity in Burbank plums stored in an atmosphere containing 50 percent carbon dioxide for 6 days at 0°, 10°, and 20° C. Solid lines, carbon dioxide treated; broken lines, control.

CAROTENE IN CARROTS

In some of the earlier experiments the treated carrots were not as yellow as the untreated. Later, samples were analyzed for carotene content. Carrots were treated with 45-percent carbon dioxide for 11 days at 20° C., 14 days at 10°, and 15 days at 0°. At the end of these periods there was no difference between treated and untreated carrots in carotene content.

[5] The following definition is quoted from Armstrong and Armstrong (2, p. 1): "The term glycoside is applied to a large number of sub stances having the property in common of furnishing a sugar and one or more other products when hydrolised by acids * * *. The term glycoside is now used officially as a general name for the group, irrespective of the sugar present; glucoside is the specific name used for those glycosides the sugar constituent of which is glucose."

SUBSEQUENT EFFECT OF CARBON DIOXIDE STORAGE

CARBOHYDRATE TRANSFORMATION IN PEAS AND SWEET CORN

Figure 8 represents changes in sucrose content of Nott Excelsior peas stored in pods for 4 days at 0°, 5°, and 25° C. The lots treated at 5° and 25° received 42 percent carbon dioxide for 2 days and were then removed from the carbon dioxide chambers and held for the remaining time at the same temperatures in air. The controls were held in air during the entire period.

Both lots at 25° C. lost sucrose, but the control lot lost it much more rapidly than the treated lot. One day after removal from storage in 42-percent carbon dioxide, the treated lot retained 60.5 percent of its original sucrose as compared with 25 percent in the control. However, the 2-day treatment was too severe from the standpoint of flavor, and the treated lot eventually lost its palatability.

FIGURE 8.—Sucrose content of Nott Excelsior peas stored 2 days in an atmosphere containing 42 percent carbon dioxide and 2 days thereafter in air, at various temperatures. Solid lines, treated; broken lines, control.

At 5° the carbon dioxide treatment showed no advantage over the low temperature for retention of sugar.

Figure 9 shows the results of a similar treatment of Golden Giant sweet corn. Treatment with 42-percent carbon dioxide for 2 days so retarded the sugar loss at 5° C. that even up to the sixth day (fourth day after removal from carbon dioxide) the treated lot retained 74.6 percent of its original sugar as compared with 48.1 percent in the control.

At the end of the 2-day treatment the lot treated at 15° C. had 44 percent more total sugar than the control. On the fourth day the two curves practically coincided, but the control lot at this temperature had lost the bulk of its sugar during the first 2 days. The carbon dioxide treatment at 25° had no effect on retaining the sugar. The flavor was satisfactory in all cases in this experiment.

RIPENING OF TOMATOES

When the carbon dioxide studies of tomatoes were inaugurated it was noticed that some of the lots that received treatment appeared not to ripen properly. This was evidenced by the failure to obtain the full red color characteristic of ripeness. Additional studies of this phase were made, including total acidity determinations.

Marglobe tomatoes in the mature green stage were stored for 2 days in pony refrigerators under the following conditions: Lot 1, pan full of ice; lot 2, 25 pounds of solid carbon dioxide, and remaining space in the ice pans filled with ice; lot 3, 12 pounds of solid carbon dioxide, and remaining space filled with ice. A fourth lot was held in the ripening room (21° C.; 85–90 percent relative humidity) during the entire time. The carbon dioxide in lot 2 attained a maxi-

mum of 44 percent within the first few hours. The percentage of carbon dioxide dropped off gradually and registered 32.6 percent at the end of the second day, when the refrigerators were opened. During this same time the carbon dioxide in lot 3 ranged from 30 percent to 14.6 percent. After the refrigerators were opened, all lots were held in the ripening room for further observations. Ten representa-

tive tomatoes from each lot were reserved for notes on color changes. Every 3 or 4 days 10 tomatoes were removed from each lot and sampled for total acidity in the pulp.

Six days after the beginning of the experiment the lot stored in the ripening room showed 5 out of the 10 tomatoes red ripe and the rest turning or yellow red. None in the other lots had advanced beyond the turning stage. Ten days after the beginning of the experiment the tomatoes stored from the beginning in the ripening room were all red ripe or yellow red. Lot 1

Figure 9.—Total sugar content of Golden Giant sweet corn stored 2 days in an atmosphere containing 42 percent carbon dioxide and 2 to 4 days thereafter in air, at various temperatures. Solid lines, carbon dioxide treated; broken lines, control.

and lot 3 had 6 and 7 red-ripe fruits, respectively, with the rest in the pink to turning stage. Lot 2, the tomatoes receiving the highest concentration of carbon dioxide, had 3 fruits in the yellow-red stage and the rest in the pink and turning stage. Three days later all save those in lot 2 were red ripe. Lot 2 never advanced beyond the yellow-red stage before
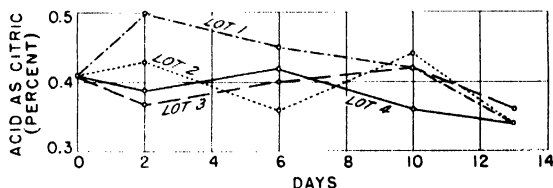
Figure 10.—Total acidity in Marglobe tomatoes stored under various conditions for 2 days and then stored in the ripening room (21° C.). Lot 1, stored 2 days in pony refrigerator containing ice; lot 2, stored 2 days in pony refrigerator containing ice and 25 pounds of solid carbon dioxide; lot 3, stored 2 days in pony refrigerator containing ice and 12 pounds of solid carbon dioxide; lot 4, stored in the ripening room (21° C.) for duration of experiment.

decay developed. Figure 10 shows the changes that took place in total acidity of the tomato pulp as a result of the different treatments. Following removal of the tomatoes from the refrigerators there was considerable fluctuation in the total acidity. At one time during the sampling period the acidity of each lot was higher than at the original sampling and at another time it was lower. At the

end of the 13-day storage period the acidity values were similar for all lots and had fallen below the original amount. Apparently the tomatoes were all physiologically ripe at the end of the experiment, even though pigment formation in lot 2 was altered if not inhibited. By the end of the experiment the flavor of all lots was considered fair.

DISCUSSION

The foregoing results indicate that the effect of storage in an atmosphere of carbon dioxide on peas and sweet corn is evident after removal from storage. However, the rate of sugar loss after removal is just as rapid in the lots treated with carbon dioxide as in the control lots, and in some instances it is more rapid, but the treated lots usually contain more sugar than the controls upon removal from the carbon dioxide. Thus the lots treated with carbon dioxide continue sweeter than the controls even though they are losing sugar rapidly after removal from the carbon dioxide treatment, and the advantage of the treatment is extended several days.

In this and in previous investigations it is shown that carbon dioxide has little effect on retaining sugar in sweet corn at 25° C. but a very pronounced effect at 5°, and that the reverse is true with peas and lima beans.

Green peas, sweet corn, and lima beans all lose their sugar rapidly at 25° C. Though similar in this respect, their response to carbon dioxide treatment indicates a physiological difference, and it would be interesting to determine whether this applies to other cereals and legumes.

The effect of carbon dioxide on carrots has been mainly to retard the conversion of sucrose into reducing sugar. Later in their storage life carrots begin converting reducing sugar into sucrose, but no attempt was made in these experiments to hold them until this stage was reached. In the present experiments the carrots treated with carbon dioxide were always sweeter to the taste and less coarse than the controls, when the carbon dioxide treatment was not continued too long. The flavor of the carrots was not affected by treatment for 4 days at 25° C., 6 days at 15°, or 9 days at 5°. Longer treatments at these temperatures usually produced a disagreeable flavor.

With the exception of peaches and cherries, the general effect of the carbon dioxide treatment on acids was to reduce the total acidity and hydrogen-ion concentration of the fruits and vegetables studied. This is in agreement with most of the work previously reported. The effect was usually most pronounced at the higher temperatures. The results were not always consistent for tomatoes at 10° and 0° C. It is probable that there is an effect of temperature that modifies any other effect. Under certain conditions storage of tomatoes at low temperatures prevents proper ripening of the fruit. In the experiments reported in this paper the period of storage may not have been long enough to interfere seriously with subsequent ripening. The normal physiological processes may have been sufficiently inhibited to produce the inconsistencies in acidity changes. Failure to color properly after removal from the refrigerators may have been due also to the high percentage of carbon dioxide.

A constant relation between total acidity and hydrogen-ion concentration was not always observed. In the studies on tomatoes the carbon dioxide treatment reduced the hydrogen-ion concentration much more consistently than the total acidity. The reverse was true of plums. Caldwell (*6*) states that there is no constant relation between total and active acidity values in fruits and vegetables, and only the broadest general parallel in their changes during ripening. The same result has been reported for vegetative parts by Gustafson (*10*). Diehl and Magness (*9*) found it difficult to obtain uniform samples for acidity determinations in plums. Allen (*1*) calls attention to the fact that the acidity in ripe plums becomes localized around the pit and in the flesh near the skin, which may influence the uniformity of the results.

Too much emphasis should not be placed upon the results on peaches and cherries as compared with those on tomatoes and plums, inasmuch as the former received but 24- or 48-hour treatments. But under these conditions there was apparently no reduction of acidity during treatment.

<p style="text-align:center">SUMMARY</p>

The results of studies made to determine the effect of carbon dioxide upon fruits and vegetables are reported.

Green lima beans and sweet corn placed in storage at 0° C. immediately after harvest required 20 and 48 hours, respectively, to attain the temperature of the storage room.

Sweet corn held for 24 hours in 37- to 50-percent carbon dioxide in pony refrigerators retained about twice as much sugar as did that stacked on the floor during the same time, but after being canned it lost some of its original fresh flavor.

Loss of sucrose was retarded in lima beans and carrots by storage in approximately 40-percent carbon dioxide. The effect was much more pronounced at 15° and 25° than at 5° C.

Carbon dioxide treatment had no effect on acidity of Early Richmond cherries in 24 hours' treatment nor on Elberta peaches in 48 hours. Exposure of Earliana and Marglobe tomatoes to 50-percent carbon dioxide for 6 days resulted in a reduction of total acidity at 20° C. and a reduction in hydrogen-ion concentration at 10° and 20°. Treatment of Burbank plums with 50-percent carbon dioxide for 6 days resulted in reduction in total acidity at 0°, 10°, and 20°, but there were no consistent changes in hydrogen-ion concentration.

Peaches and carrots were analyzed for glycosides following carbon dioxide treatment, but no significant quantities of these substances were obtained. There were no significant differences in the benzoic acid content of treated and untreated Burbank plums.

Treatment of carrots with 45-percent carbon dioxide for 11 days at 20° C., 14 days at 10°, and 15 days at 0° did not reduce the carotene content.

Carbon dioxide had no permanent effect on carbohydrate transformation in peas and corn, except that for several days after exposure the treated lots still contained much more sugar than the controls.

Pony-refrigerator experiments with Marglobe tomatoes indicate that, although too high a concentration of carbon dioxide during storage may retard pigment formation during subsequent ripening,

the acidity changes are similar to those in the untreated lots and the reduction in acidity accompanying ripening is not inhibited by the carbon dioxide treatment.

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ABSORPTION, DISTRIBUTION, AND SEASONAL MOVEMENT OF POTASSIUM IN YOUNG APPLE TREES AND THE EFFECT OF POTASSIUM FERTILIZER ON POTASSIUM AND NITROGEN CONTENT AND GROWTH OF TREES [1]

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INTRODUCTION

In certain orchard investigations (8) [3] begun in 1928 at the University of Maryland, which involved a study of annual surface applications of potassium fertilizers to bearing apple and peach trees, no response in growth or fruiting was apparent even after several seasons. The question then arose as to whether there was an actual increased intake of potassium by fruit trees so treated under field conditions. The logical approach to the problem lay in comparing the chemical composition of trees treated with potassium fertilizers with those not so treated. To facilitate the study young trees were used. However, the same method of surface application of potassium fertilizer followed under ordinary field conditions was employed in the nursery row. Thus the results should be comparable to those of field studies with older trees. By studying the seasonal chemical changes in various fractions of these young trees, it was planned (1) to determine the period and rate of absorption of potassium; (2) to ascertain the relative concentration of potassium in the different parts of the tree, as well as the actual amounts present; (3) to obtain an indication as to any seasonal movements of potassium from one tree part to another; (4) to determine the effect of heavy applications of potassium fertilizer on the relationships stated above; (5) to study the effect of heavy applications of potassium fertilizer on the total nitrogen content of the tree parts; (6) to investigate the association of nitrogen with potassium in the tree parts throughout the season; and (7) to determine any growth responses caused by potassium fertilizer.

LITERATURE REVIEW

Working at New Hampshire in 1916 and using 7-year-old Golden Ball apple trees, Butler, Smith, and Curry (5) found that there was little movement of potassium until about the period of bloom, when there was a translocation from the older branches to the younger twigs. The young roots decreased in percentage of potassium from the dormant period until the time of bloom (May 18). From then until active growth ceased (July 12) the concentration increased

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markedly, and then fell off again. The leaves were not included in this study.

Richter (*13*), working in Germany about 1909, studied the potassium content of apple leaves throughout the growing season, and found that on a percentage basis the potassium content decreased as the season advanced. On an absolute basis, a maximum was reached at the stage of maximum leaf size. Roberts (*14*) in 1895, Van Slyke et al. (*19*) in 1905, and Thompson (*18*) in 1916 all attempted to interpret the fertilizer needs of fruit trees by chemical analysis of the entire tree and a calculation of the total amount of any essential element removed over a definite period. Their work yielded no additional information as to seasonal movements of potassium, but it did give an indication as to where potassium was located in the apple tree. Since Roberts worked with a nonbearing tree, even though it was mature, some of his data recalculated to show the relative proportions of the potassium located in the different parts of the tree, are presented. The analysis showed that 15.0 percent of the tree's potassium was located in the leaves, 7.1 percent in the twigs of the current season, 9.0 percent in the 1-year twigs, 58.3 percent in the trunk and limbs, and 10.5 percent in the roots.

Burke and Morris (*4*) in Montana studied the movement of potassium from the tree into the new growth and leaves of 20-year-old apple trees. One tree was sampled during the dormant period (Apr. 9) and another when the leaves had attained full size (June 11). By comparing the amount of potassium in the dormant tree with that in the active tree they could calculate the extent of movement up from the soil and roots, as well as the movement from the old wood into the new growth and leaves. They found that potassium moved upward from the roots, trunk, and large limbs. There was sufficient movement from the soil and roots, however, to supply the needs of the leaves and new twig growth. Unfortunately, they did not dig the entire root system and therefore could not differentiate between potassium coming from the soil and that coming from the roots.

MATERIALS AND METHODS

DESCRIPTION OF PLOTS

From a group of 400 2-year-old Stayman Winesap apple trees growing in the nursery row, on a Sassafras sandy loam at College Park, Md., 190 of the more uniform [4] were selected for the experiments. The block of trees consisted of 4 rows about 4 feet apart, the trees being approximately 18 inches apart in the row. The trees were allowed to remain in their original places.

The block was divided into four plots, each of which received an application of one-fourth pound of ammonium sulphate and one-half pound of superphosphate per tree. Two of the plots received an application of 1 pound per tree of sulphate of potash. This was considered to be a heavy application of potassium.[5] The materials were applied April 1, 1933.

[4] The coefficient of variability of the diameters of the 190 trees was 1.72 percent.

[5] Replaceable potassium determinations of the surface 18 inches of soil were made before the fertilizers were applied and at intervals throughout the growing season. The results indicated that the normally treated plots remained at about 34 p. p. m., while the potassium-treated plots increased to about 540 p. p. m. after treatment and remained at approximately that figure throughout the season.

The trees had been planted on a gentle slope with the rows set in the direction of the slope. The plot boundaries were perpendicular to the rows, so that each plot contained a section of all rows. Since not all trees in the rows were used, the number of trees in the plots was unequal. However, the number present in each plot was sufficient to be representative of the treatment. The plots were numbered from 1 to 4, going up the hill, 1 and 3 receiving the potassium.

GROWTH RECORDS

Since it was desired to study the effect of the heavy application of potassium upon the growth of the trees, records were taken at weekly intervals during the greater part of the growing season, although occasionally a longer interval between measurements was necessary.

The diameter at a marked point was taken on each measuring date, the accuracy of the measurement extending to 0.01 cm. The longitudinal growth of the terminal shoots of each tree was measured in centimeters.

SAMPLING METHODS

Samples for chemical analysis were secured at 12 intervals between April 8, 1933, and January 8, 1934, inclusive. Since the trees had not previously exhibited any potassium-deficiency symptoms, the treatment involving only nitrogen and phosphorus was considered to be normal, and since a study under normal conditions was desired, four trees were taken as a composite sample from these plots on each sampling date. From the plots receiving a heavy application of potassium only a single tree was sampled on each date, the assumption being that if any marked compositional differences between the potassium-treated and the untreated trees existed, they could be detected by the analysis of the one tree. Furthermore, it was believed that the variability among the normally treated trees probably would represent the variability which would be expected from the trees receiving the potassium fertilizer. The trees dug on any sampling date were always selected at random, except that no two adjacent trees were used for analysis, thus avoiding any possible injury to the root systems.

The entire trees were dug, care being taken to preserve as many roots as possible. However, during the latter part of the season, when the root systems were rather extensive, some of the finer roots were lost. The trees were dug between 7 and 8:30 a. m., following a sunny day. They were never dug when rain was falling, although in a few instances rather cloudy weather prevailed. The digging was done as rapidly as possible and the trees were carried to the laboratory for subsequent treatment.

After the trees had been washed and weighed, they were separated into 18 different fractions. (There were fewer fractions during the early part of the growing season or when no leaves were present.) The following fractions were secured: Leaves, 1933 upper bark, 1933 upper wood, 1933 lower bark, 1933 lower wood, 1932 upper bark, 1932 upper wood, 1932 lower bark, 1932 lower wood, 1931 upper bark, 1931 upper wood, 1931 lower bark, 1931 lower wood, large root bark, large root wood, small root bark, small root wood, and rootlets.

The fractions were designated in accordance with the year of their first longitudinal extension. The upper and lower classification

designated the upper and lower half of each section. The large roots were those 1 cm or more in diameter; the small roots ranged from 1 cm to 2 mm in diameter; the remainder of the root system was designated as rootlets.

Since considerable time was required to fraction five trees, the bark/wood ratio was obtained from the tree fractioned for moisture determinations. (See under Chemical Methods.) Then the weights of the fractions, including bark and wood for all trees, were recorded and the dry weights of the individual fractions later were calculated from the bark/wood ratio and moisture content of the one tree.

Smaller samples, taken from the composite sample of the four trees from the no-potassium plots, were peeled, dried, and ground for chemical analysis.

All leaves were picked off and thoroughly mixed. From this lot about 200 leaves were selected at random to constitute the sample for analysis.

CHEMICAL METHODS

Moisture.—On the first three sampling dates the moisture content of all fractions of trees from both treatments was obtained, but inspection of the results showed that the trees sampled first always had a slightly higher moisture content, regardless of treatment. Therefore, on subsequent sampling dates the one tree from the high-potassium plots was immediately cut up for moisture determinations, so as to avoid unnecessary water losses. All moisture calculations were based on this one tree. The samples were dried at 72° C for 48 hours in a forced-draft oven and loss in weight was called moisture.

Nitrogen.—Total nitrogen was determined by the official Kjeldahl-Gunning method (*2*).

Potassium.—Two-gram samples of the ground, oven-dried material were ashed in an electric muffle furnace at 550° C. The ash was taken up with hydrochloric acid, transferred with water to a 100-ml volumetric flask, made to volume, and a 25-ml aliquot was taken. Potassium was then determined by the sodium cobaltinitrite method of Schueler and Thomas (*16*). Since the method used had not been accepted by chemists in general, it seemed advisable to check it with the official platinic chloride method. Through the courtesy of Dr. Lilleland, of the University of California, samples of prune leaves on which determinations had been run by the official method were obtained for analysis. Table 1 gives a comparison of the two methods as evidenced by the potassium content of the prune leaves.

TABLE 1.—*A comparison of the total potassium (as percentage of dry matter) content of prune leaves as determined by the platinic chloride and sodium cobaltinitrite methods*

Sample no.	Platinic chloride method	Sodium cobaltinitrite method
	Percent	*Percent*
	1.46	1.50
	1.31	1.29
	2.31	2.38
	2.85	2.99

The figures are in rather close agreement, considering the fact that different glassware, solutions, and operators, as well as different chemical methods, were employed in obtaining the results.

EXPERIMENTAL DATA

ABSORPTION OF POTASSIUM BY THE TREES

Since some variability existed among the trees, it was necessary to smooth out the dry weights of all fractions by means of a three-figure moving average. Since a moving average results in a reduction in the number of figures, the first two sampling dates were averaged for the first figure and the dry weight for the last sampling date was left unchanged; that is, the first corrected dry-weight figure was an average of the first two, the second corrected figure was an average of the first three figures, the third an average of the second, third, and fourth figures, etc. The next to the last corrected figure was an average of the last three, and the last corrected figure was the same as the last actual dry-weight figure. After the corrected dry-weight figures were obtained they were multiplied by their corresponding potassium percentages, which gave the actual grams of potassium present in each fraction (table 2).

TABLE 2. Total amount of potassium per tree under the two fertilizer treatments, both including and excluding the leaves, 1933

Date sampled	Potassium-treated [1][2]		No potassium [2]		Physiological state of trees
	Including leaves	Excluding leaves	Including leaves	Excluding leaves	
	Grams	Grams	Grams	Grams	
Apr. 8	0.9348	0.9348	0.8669	0.8669	Buds beginning to swell; bark sticking.
Apr. 25	1.0781	.8448	.9945	.7612	Terminal buds bursting; some leaves ¼ inch long; bark still sticking on roots.
May 11	1.5019	1.0542	1.4908	.9726	Buds fully opened; in some cases very slight terminal growth.
May 29	2.6898	1.5998	2.0502	1.2462	New growth 18-22 cm long.
June 13	4.8985	2.4695	2.6993	1.5120	Terminal growth 25-40 cm; lower part of 1933 growth turning brown.
June 25			3.5938	1.8541	Terminal growth 38-48 cm; trees still growing rapidly.
July 23	6.0350	2.8332	3.5368	1.9818	Terminal growth 50-70 cm; trees healthy and vigorous.
Aug. 18	5.8268	3.2275	4.0507	2.3055	Bark sticking on few upper twigs; growth slowing down.
Sept. 10	6.5827	4.0454	4.2655	2.6027	Some leaves falling; bark sticking on all parts of tree except trunk and roots.
Oct. 4	6.0751	4.1834	3.1832	2.3303	Many older leaves fallen; bark sticking on all parts of tree.
Nov. 3	5.2806	4.4887	3.6208	3.1518	Only a few of younger leaves left; some still green.
Jan. 8		4.3528		3.2686	Tree entirely dormant.

[1] Treated with 1 pound potassium sulphate in April.
[2] All trees received a basic application of $(NH_4)_2SO_4$ and superphosphate.

It happened that the four trees of the sample taken from the normally treated plots on October 4 were rather small. Even though their low dry weights were increased by the moving average, the amount of potassium in the trees was still low, indicating that the concentration was lower than would be expected at this time of the year. The potassium values obtained on October 4 are not plotted on the graphs because the trees were considered as abnormal.

No potassium-treated tree was dug on June 25.

In connection with table 2, a note as to the approximated physiological status of the trees at each sampling date is appended. When comparisons are made between the results of this investigation and those of similar studies, the use of the tree condition, rather than the calendar dates, would seem advisable. However, in this report, for the sake of brevity, all references to time of sampling are made in terms of the actual date. Since the sampling dates were arbitrarily
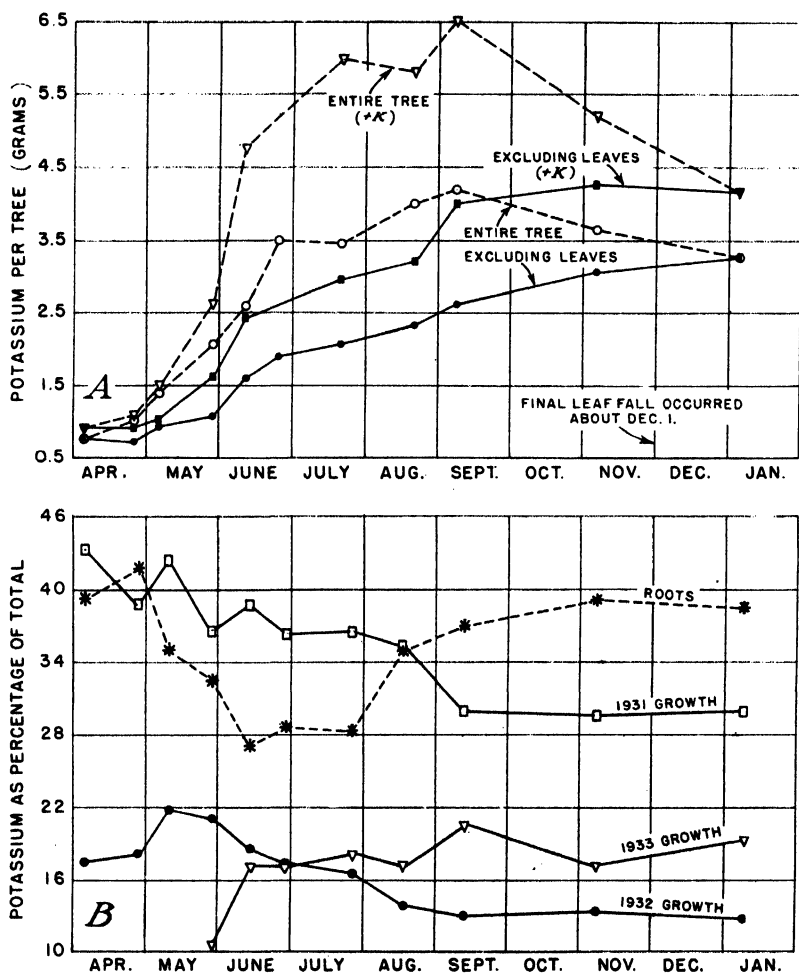
FIGURE 1.—Potassium content of: *A*, Trees, including and excluding leaves, throughout the season, under two different fertilizer treatments, with and without potassium. *B*, Different-aged portions of trees throughout the season. +K treated with 1 pound potassium sulphate in April; others untreated.

chosen without reference to any expected changes in potassium behavior, any difference between two dates may not represent the extent of the actual change which occurred.

Table 2 shows the total grams of potassium in the apple trees on the various sampling dates. Figure 1, *A*, presents the same material graphically. The data are given both for the entire tree and the tree excluding the leaves, so that the potassium content of the tree can

be studied without the introduction of the rather large amount of potassium which accumulates in the leaves, a large part of which is lost at leaf fall.

Considering both fertilizer treatments, it is evident that potassium was absorbed slowly between April 8 and 25. A portion of the potassium in the woody part of the tree moved into the leaves during this period, as is indicated by the fact that the potassium content of the tree, minus the leaf content, decreased.

From April 25 to May 11, the intake of potassium was sufficient to cause an increase in all parts of the tree, and the rate of absorption continued rather uniformly from May 11 to September 10. Leaf fall was beginning by the latter date, and from then on the potassium content of the tree as a whole decreased. However, excluding the leaves, the potassium in the tree continued to increase at a uniform rate until November 3. This increase may have resulted from autumnal migration from the leaves, but limited data which will be presented later indicate that the probability of absorption from the soil is greater. From November 3 until January 8 there was little change.

The absorption curve for the potassium-fertilized trees (+K) was similar to that for the normal treatment except that considerably more potassium was absorbed over the same period of time. Considering the large differences in potassium intake and the lack of differences in growth response of the two groups of trees, there can be no doubt that luxury consumption of potassium occurs with the apple.

SEASONAL MOVEMENT OF POTASSIUM IN DIFFERENT TISSUES AND PARTS OF TREE

In considering the seasonal movement of potassium in the different portions of the tree, each of the 18 fractions might be considered individually. However, to simplify the problem, only the different-aged portions of the tree are considered separately.[6] The fact cannot be disregarded that the growth laid down the previous season, and designated here as 1932 growth, has within it new tissues added in 1933. These tissues are probably of entirely different composition from those formed the previous year. Therefore, if tissues decrease in potassium with age and young tissues high in potassium are being deposited, the interpretation of the results is somewhat difficult. Some experimental evidence obtained on this point will be presented later. Meanwhile, the data for the potassium content of the tree fractions will be presented, disregarding the fact that the 1931 wood, for example, contains new wood added in 1932 and still younger tissues laid down in 1933.

The question arose as to whether the percentage of fresh-weight or the percentage of dry-weight figures should be used in interpreting the relative potassium concentrations in the different fractions. An inspection of the fresh- and dry-weight figures for the entire tree indicated that the dry weights presented a more stable basis on which to calculate the results. Dry matter accumulated constantly throughout the season. The fact that the fresh weights fluctuated considerably, even though the general trends were similar, indicated changes

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[6] The complete data for the individual fractions are on file at the Maryland Agricultural Experiment Station.

in moisture content during the different periods rather than any appreciable change in dry matter.

Another problem which arose in connection with the study of the potassium status of the fractions was how to consider the leaves in connection with the other tissues. They are not a permanent part of the tree, are extremely vegetative, and contain a large proportion of the total potassium in the tree. Because of these conflicting features, the leaf data are presented separately.

PROPORTIONATE DISTRIBUTION

Figure 1, *B*, presents graphically the potassium content of the different aged portions of the trees, expressed as percentage of the total. These data give an indication as to what portions of the tree contain the bulk of the potassium on the various sampling dates.

The roots at first carried 40 percent of the total potassium, but by June 13 they carried only 27 percent. In October they contained about the same proportion of the total that they had at the beginning of the growing season.

There was a general tendency for the 1931 and 1932 growths to lose in their proportionate amounts of potassium from May until September.

The 1933 growth tended to increase in its proportional amount of potassium. This might be expected since new tissues were being added throughout the season.

Averages for the entire season show that the bark of the tree under the normal treatment contained 47.5 percent of the total potassium, while the wood contained 52.5 percent, but under the potassium treatment 57.0 percent of the potassium was in the bark and only 42.1 percent in the wood. This would indicate that luxury consumption takes place more readily in the bark than in the wood. Other figures will bring this out more emphatically.

ABSOLUTE DISTRIBUTION

Considering the same material expressed in grams per fraction, the actual movement of potassium from one part of the tree to another can be traced. These data, presented in figure 2, *A*, show that the different-aged parts of the tree continually gained in potassium. The roots did not increase in potassium during the period of early growth activity at a rate similar to that of the other fractions, but from July 23 on through the season their potassium content increased greatly.

The fact that the potassium content of the 1933 growth increased more rapidly than that of the 1932 growth might be attributed to the greater proportion of young tissue in the 1933 growth. Dowding (9) and many others have reported a high concentration of potassium in the younger tissues. This theory, however, does not hold true when the 1931 growth is considered, since its increase is more rapid then either of the younger tissues.

The total amount of potassium in the 1931 and 1932 growth followed closely the dry weight, as figure 2, *A*, and *B*, shows. The roots and new growth, however, increased in potassium during the latter part of the growing season more rapidly than they accumulated dry matter.

CONCENTRATION

The amount of potassium in each fraction was expressed as percentage of the dry matter. The results are shown in figure 3, *A*. The data indicate that the concentration of potassium in the new growth was very high at the start of the season as compared with that in other portions of the tree, but it decreased suddenly between May 29
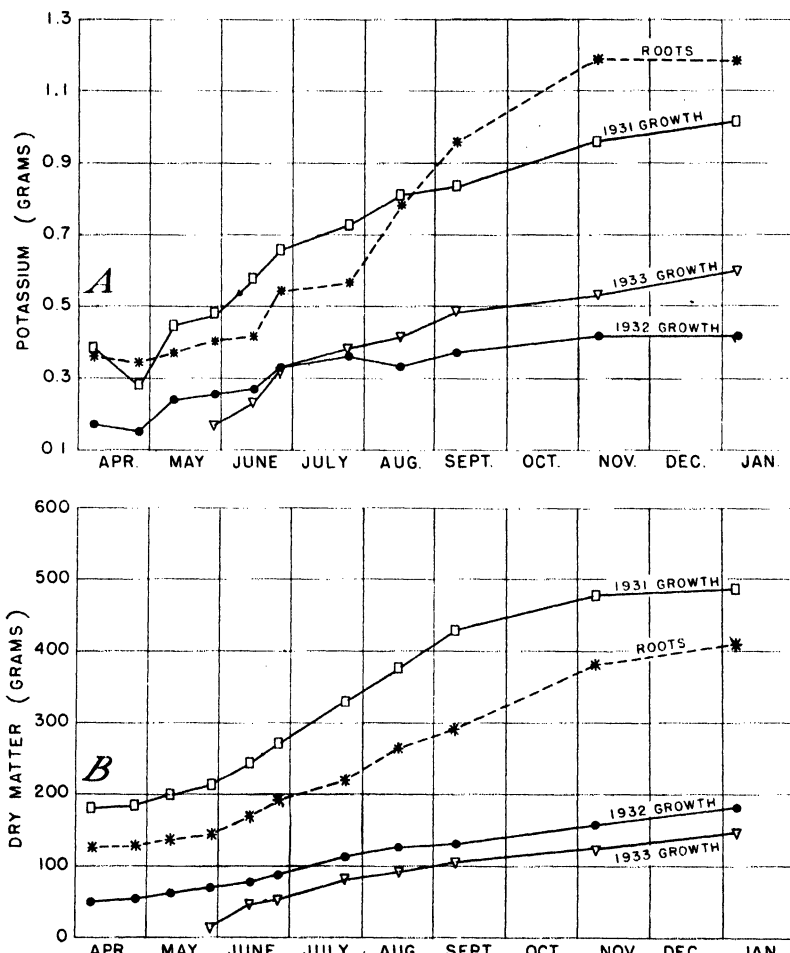
FIGURE 2.—Absolute amount of potassium (*A*) and of dry matter (*B*) in the different aged portions of the trees throughout the season.

and July 23. After that it decreased gradually throughout the growing season. The concentration of potassium in the 1932 growth, after May 29, showed a gradual decrease throughout the season, while that in the 1931 wood remained fairly constant, decreasing only slightly.

The potassium content of the roots remained rather constant until the latter part of July, when it increased. The increase continued until September 10, and the potassium remained high throughout the rest of the sampling period.

It is evident that the percentage composition is an expression of the relation between the absolute increase in potassium and the increase in dry matter. If dry matter and potassium increased proportionately, the percentage composition would remain the same. However, as the graphs bear out, in the roots the increase in potassium was greater than the increase in dry weight, while in the 1933 growth the reverse was true.
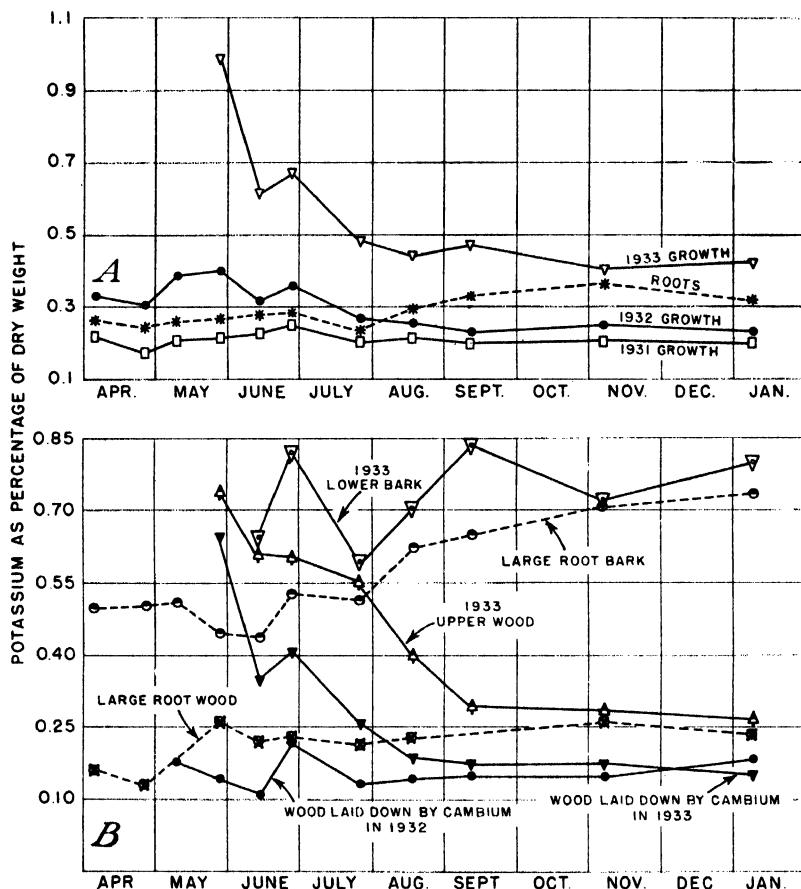
FIGURE 3.—Potassium content of: *A*, Different-aged portions of the trees; *B*, certain bark and wood fractions.

DISTRIBUTION BETWEEN BASAL AND APICAL PORTIONS OF GROWTH AND AMONG TISSUES

The question now arises, did both basal and apical portions of the different-aged parts of the tree act similarly throughout the season? Certain unpublished data indicate that, on a percentage basis, the bark and the wood, both upper and lower, of the 1932 and 1931 growth did act very similarly, but in the other parts differences existed.

The trends for potassium, as percentage of dry weight of the upper 1933 wood and lower 1933 bark, as well as those for the large root bark and root wood are presented in figure 3, *B*.

The large increase in the roots after July 23 was caused principally by increases in the bark rather than in the wood.   The root bark and wood seemed to exhibit a somewhat reciprocal relationship during the early part of the growing season, thus accounting for the constant percentage composition of the roots as a whole.   The causal factors are unknown.   Figure 3, *B*, brings out the fact that the decrease in percentage composition of the 1933 growth was due largely to decrease in the wood fraction, since the bark showed no material decrease.

On an absolute basis the bark and wood fractions, both upper and lower, maintained trends similar to those of the entire season's growth (bark and wood, upper and lower combined), the only differential response being a relatively faster increase in potassium in the bark than in the wood.   These statements apply to all sections of the tree regardless of age.

Certain unpublished data indicate that the old wood is relatively inactive with respect to potassium changes.   Of course, if potassium
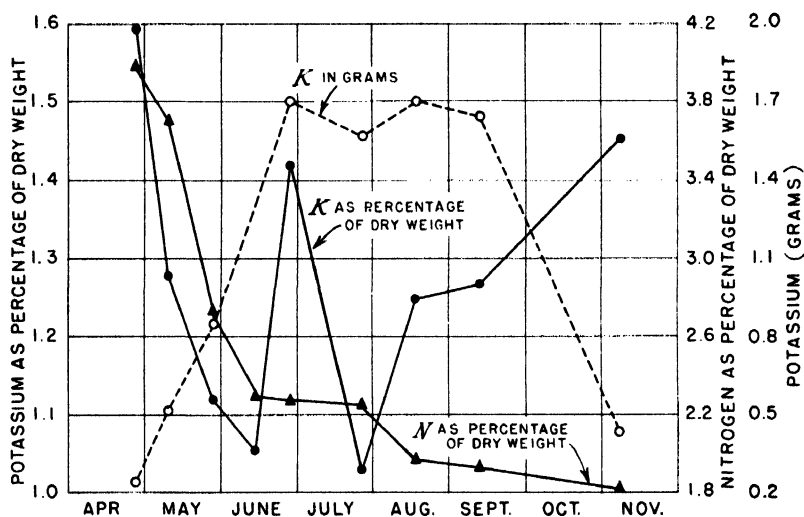
FIGURE 4.—Potassium content of the leaves throughout the season; also nitrogen content.

were merely passing through the conducting elements of the xylem, marked seasonal differences would not be expected.   However, the new wood laid down by the cambium should be relatively high in potassium if the number of living cells in a tissue is correlated with its potassium content.   In order to study this point, the new wood laid down on the lower 1932 section of the tree was separated on each sampling date and subsequently analyzed for potassium.   The curves, as percentage of dry matter, are plotted in figure 3, *B*.

The curve for the new wood on lower 1932 growth (wood laid down by cambium in 1933) very closely resembles that for the upper 1933 wood.   This appears to indicate that the decrease in concentration of potassium in new wood was similar whether it was initiated from the cambium or was a combination of an apical meristem and the cambium.   The old wood laid down in 1932 did not show any marked change in potassium concentration throughout the season.

POTASSIUM CHANGES IN THE LEAVES

The leaves, at their maximum weight, contained 44 percent of the total potassium in the tree. The fact that the seat of photosynthetic activity is in the leaves may be associated with this high concentration, since potassium is considered to play a part in carbohydrate metabolism.

Figure 4 shows the potassium composition of the leaves on a percentage dry-weight and absolute-amount basis throughout the season, and the nitrogen composition as percentage of dry weight.

The curves indicate that on a percentage basis the potassium content of the leaves fell rapidly until June 13, rose sharply until June 25, then declined until July 23. From that time until leaf fall the concentration of potassium increased. The sudden rise between June 13 and June 25 cannot be explained. It is doubtful whether sampling error could account for it. The chemical determination was checked carefully.

On an absolute basis the potassium content increased until June 25, remained fairly constant until September 10, and then fell off as leaf fall began. The curve is such as one would expect since it conforms quite well with the total dry weight curve of the leaves.

The curve for nitrogen content in the leaves expressed as percentage of dry weight presents a picture quite different from the corresponding curve for potassium. The concentration of nitrogen decreased continually throughout the season, although rather slowly during the latter part of the growing period. The fact that the potassium concentration increased so strikingly during the autumn when the nitrogen concentration decreased, suggests the unlikelihood of an autumnal migration of potassium into the bark and wood. However, the fact that the older leaves fell before the younger ones tended to cause the potassium and nitrogen content of the leaves to be high at the close of the season and may have obscured some autumnal migration.

To substantiate the fact that young leaves contain more potassium than older ones, on three sampling dates the leaves growing on the upper end of the current season's growth were separated from those growing on the lower portion. The results are reported in table 3.

TABLE 3.—*Potassium content of old and young leaves at 3 sampling dates, 1933*

Date	Leaves	Potassium as percentage of dry matter	Potassium per 100 leaves
		Percent	*Grams*
July 23	{Young	1.539	0.529
	{Old	1.102	.337
Aug. 16	{Young	1.632	.614
	{Old	1.320	.459
Sept. 10	{Young	1.639	.590
	{Old	1.293	.383

RELATIVE POTASSIUM CONTENT OF THE VARIOUS FRACTIONS

Obviously the various fractions differed in their potassium content on the different sampling dates. However, certain relationships among the different parts held true generally throughout the season.

Figure 5 gives the average potassium content of the various fractions, samples from all dates being averaged.

On a percentage basis the leaves were the richest in potassium. As percentage of dry matter, there was a decreasing gradient of potassium in the bark from the apical end of the tree to the smallest roots. In the wood there was a similar decrease in potassium until the base of the tree was reached, but there the large roots showed

FIGURE 5.—Potassium content of the different fractions of the trees expressed as: *A*, Percentage of dry weight; *B*, as grams per fraction. Averages for entire season.

some increase in potassium concentration and the small roots even more.

A comparison of percentage results with the absolute amounts of potassium in the tissues (fig. 5, *B*) shows the leaves to be higher on both bases. With the exception of the 1933 bark, there was an increasing gradient of potassium on the absolute basis down the tree to the small root bark. Thus the absolute amounts were generally

negatively correlated with the percentage composition. The wood generally exhibited an increasing amount of potassium from terminal portion to root portion of the trees. The small roots were exceptional, and the 1933 lower wood was somewhat higher in potassium than the 1932 wood. However, in general, both with bark and wood, where the concentration of potassium was highest, the absolute amounts
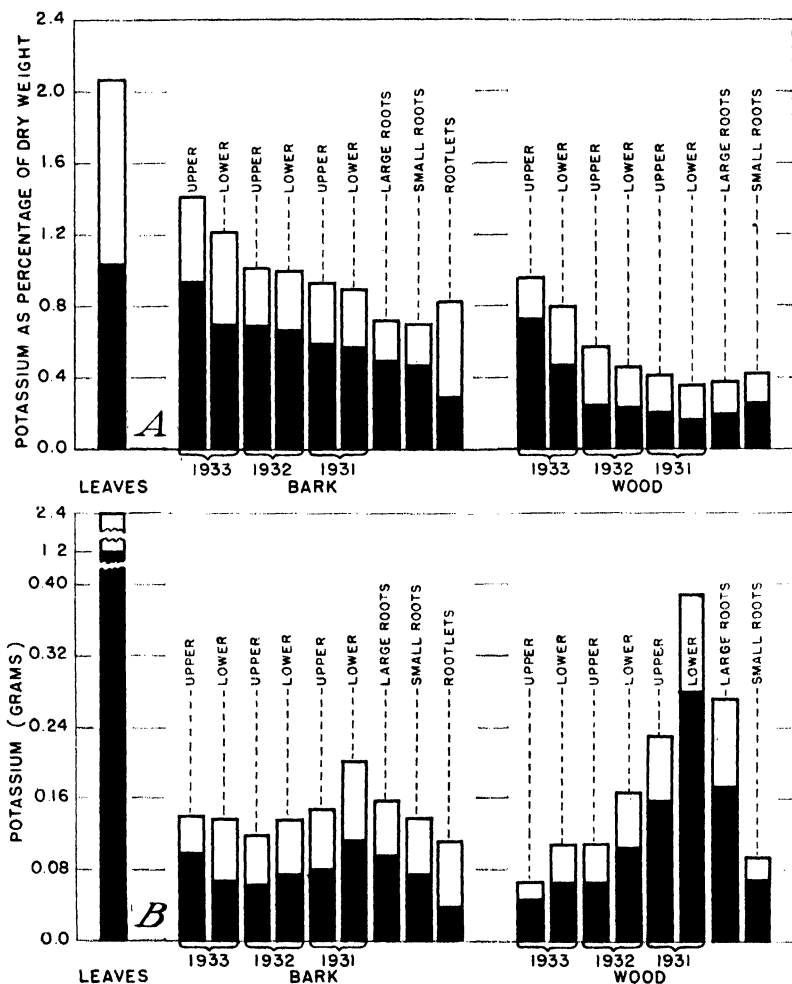
FIGURE 6.—Potassium content of the various fractions of trees on June 13, comparing: *A*, The potassium-treated trees with the normally treated trees; *B*, the treated trees with the normal ones. The black part of the bars denotes potassium content under normal treatment; the white part shows accumulation due to fertilizer application.

were lowest. The most logical explanation of this would be that potassium is highly concentrated in young, active tissues, while the bulk of the tree, being made up of woody structural material, has a low concentration of potassium. This point has not received proper consideration in the literature, many investigators reporting that most of the potassium was located in the bark.

Although there were interactional effects between sampling dates and fractions with respect to potassium content, the various fractions showed approximately the same relationships throughout the season.

EFFECTS OF HEAVY APPLICATIONS OF POTASSIUM FERTILIZERS ON POTASSIUM CONTENT AND DISTRIBUTION

Figure 1 showed that potassium had entered the tree in considerably greater amounts under heavy applications than under normal treatment.   Figure 6, *A*, shows the potassium concentration in the tree tissues on June 13 for both the potassium-fertilized and the normally treated trees.   The potassium content of the leaves increased considerably more than that of either the bark or the wood.

A similar relationship existed in the absolute amounts (fig. 6, *B*), the potassium content of the leaves increasing about 100 percent as a result of the treatment.   The same general tissue relationships are
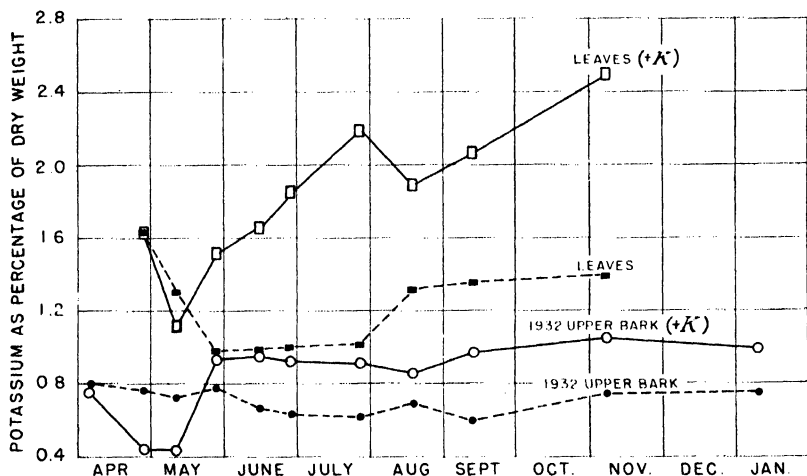
FIGURE 7.—Potassium content of the leaves and the 1932 upper bark to show the period when the increased potassium content of the potassium-fertilized trees became noticeable.   +K trees treated with 1 pound potassium sulphate per tree in April; other untreated.

apparent here as in figure 5 except that the root bark was relatively low in potassium.   It will be recalled that the increased movement of potassium into the roots did not take place until after June 13.

Apparently between 1 and 2 months were required for the applied potassium to enter the tree and move up to the leaves and upper twigs.   Figure 7 shows the seasonal trend of the leaves and 1932 upper bark for both treatments.   In both cases the treated (+K) and normal curves cross between the May 11 and May 29 sampling dates.   From this period on the leaves from the trees treated with potassium fertilizer always maintained a higher concentration of potassium.

All tissues exhibited luxury consumption, but the leaves showed it to a greater degree than any other part of the tree.   The leaves from the +K treatment abscissed somewhat earlier in the autumn, indicating that certain toxic, or at least detrimental, effects occurred from the heavy application of potassium.

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EFFECT OF POTASSIUM APPLICATIONS ON TOTAL NITROGEN CONTENT OF TREES

Table 4 shows the total nitrogen content of the trees under both treatments throughout the season, including and excluding the leaves. Fisher's *t* comparison (*10*) made between the nitrogen
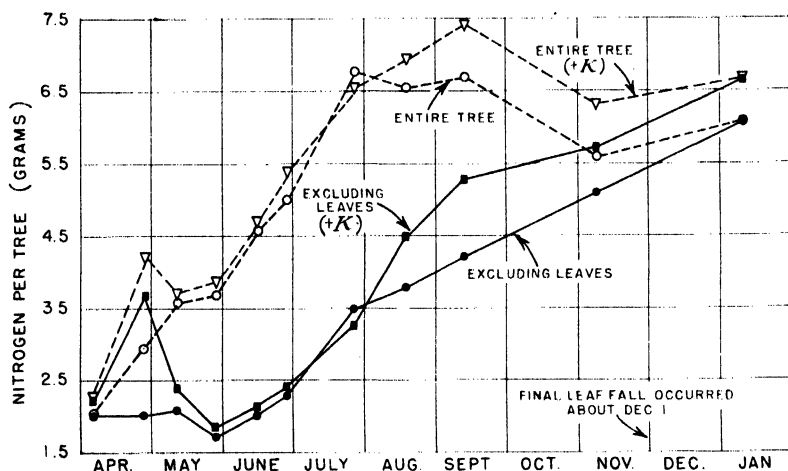
FIGURE 8.—Nitrogen content of potassium-fertilized as compared with that of normally treated trees, including and excluding leaves. +K trees treated with 1 pound potassium sulphate in April; others untreated.

TABLE 4.—*Total amount of nitrogen per tree under the two fertilizer treatments, both including and excluding the leaves, 1933*

Date sampled	Potassium-treated [1] [2]		No potassium [2]	
	Including leaves	Excluding leaves	Including leaves	Excluding leaves
	Grams	*Grams*	*Grams*	*Grams*
Apr. 8	2.3384	2.3384	2.1233	2.1233
Apr. 25	4.3412	3.7627	2.7101	2.1315
May 11	3.7784	2.4508	3.6989	2.1489
May 29	3.8362	1.9130	3.8050	1.8281
June 13	4.9359	2.1620	4.6502	2.0048
June 25			5.0258	2.2919
July 23	6.6399	3.4230	6.8996	3.4856
Aug. 16	7.1106	4.2675	6.5503	3.8200
Sept. 10	7.7134	5.2495	6.7189	4.2601
Oct. 4	6.7449	6.0522	6.4094	4.7159
Nov. 3	6.4028	5.7405	5.6270	5.0390
Jan. 8		6.7755		6.3959

[1] Treated with 1 pound potassium sulphate Apr. 1.
[2] All trees received a basic treatment of $(NH_4)_2SO_4$ and superphosphate.

content of the trees from the two treatments shows that the heavy application of potassium caused the total nitrogen content of the trees to be significantly increased. Disregarding nitrogen lost at leaf fall, the trees receiving an application of potassium absorbed 4.4371 g of nitrogen while the normally treated trees absorbed only 4.2746 g. This increase, expressed as the average of all sampling dates, is 17.7 percent. The material is presented graphically in figure 8. That the curves for the nitrogen content of the treated and untreated trees move further away from each other as the season

advances also indicates that the difference was due to treatment rather than to chance. This agrees with the work of Gildehaus (*11*) which showed that a high concentration of potassium in the culture solution resulted in a higher nitrogen content.

In an effort to determine what tissues were responsible for this phenomenon, the differences for each tissue, averaging all sampling dates, were calculated, and expressed as percentage of the nitrogen content (absolute amount) of the untreated trees. This material is presented in table 5.

TABLE 5.—*Comparison of the average nitrogen content for entire season of different fractions of trees fertilized with potassium sulphate with that of trees denied potassium*

Tissue	Treated minus untreated	Increase (+) or decrease (−)	Tissue	Treated minus untreated	Increase (+) or decrease (−)
	Grams	*Percent*		*Grams*	*Percent*
Leaves	−0.018	−0.9	Upper 1931 bark	−.001	−0.7
Upper 1933 bark	.062	35.0	Upper 1931 wood	.021	8.8
Upper 1933 wood	.053	46.9	Lower 1931 bark	.008	4.1
Lower 1933 bark	.023	10.1	Lower 1931 wood	.091	19.5
Lower 1933 wood	.180	109.7	Large root bark	.026	12.3
Upper 1932 bark	.004	3.4	Large root wood	.013	2.0
Upper 1932 wood	−.001	−6.8	Small root bark	.038	21.0
Lower 1932 bark	−.005	−4.0	Small root wood	.012	4.9
Lower 1932 wood	.016	10.4	Rootlets	−.004	−3.2

Any exact statement concerning the tissues is impossible. The current season's growth, however, showed the greatest difference, with the root bark next in order of importance. In general, except in the roots, the wood exhibited greater differences than the bark. There was essentially no difference between the leaf tissues. The importance of this relationship could hardly be conjectured, though it might designate some relationship between potassium supply and protein synthesis.

ASSOCIATION OF POTASSIUM WITH NITROGEN IN THE VARIOUS TISSUES THROUGH OUT THE SEASON

Nitrogen enters into many organic compounds in the plant, while potassium is believed to be largely in solution as inorganic salts. However, both elements are associated with living tissue, and it is of interest to compare their behavior in apple trees.

The total absorption curves of potassium and nitrogen have already been presented in figures 1 and 8 respectively. Comparing the normal treatment in both cases, the curves are found to be similar, with two exceptions, (1) although nitrogen and potassium moved into the leaves rapidly during the latter part of May, absorption of nitrogen from the soil was much slower at that time; (2) nitrogen increased from November 3 to January 8 while potassium remained rather constant This latter result seems significant, although it would require duplication before it could be deemed valid.

Figure 9, *A*, shows the percentage of the total nitrogen present in the different-aged portions of the tree and in the roots throughout the season. The data are rather similar to the corresponding potassium data shown in figure 1, *A*, the only exceptions being that a larger proportion of nitrogen resided in the roots than in the 1931 growth
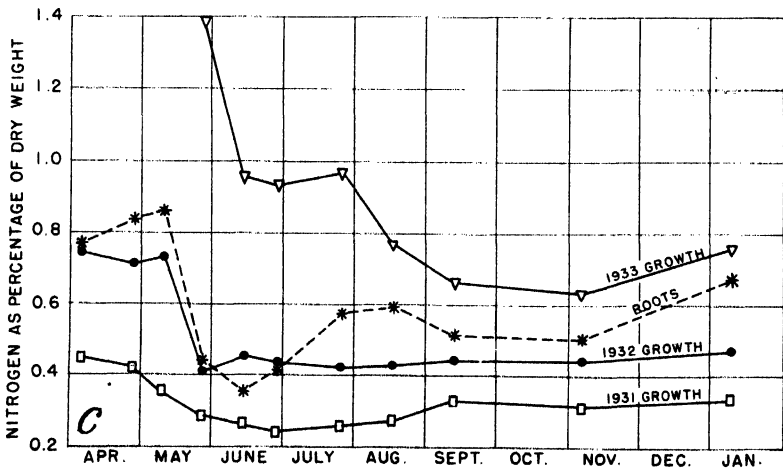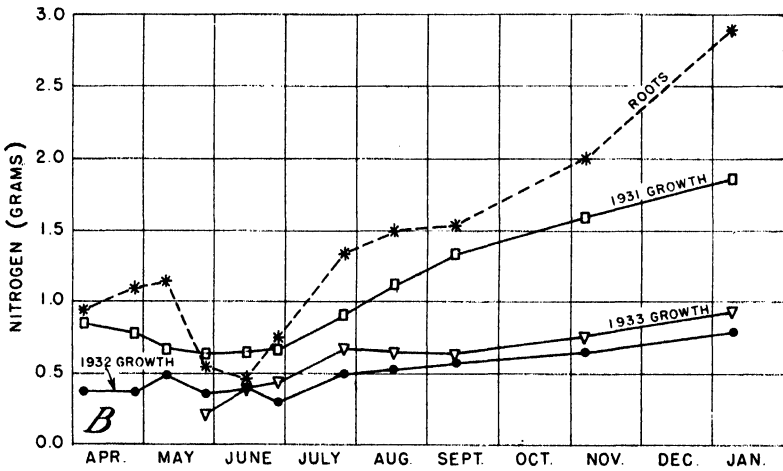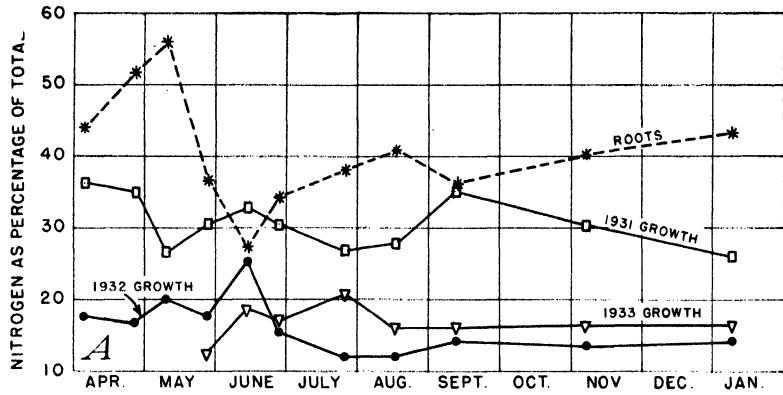
FIGURE 9.—Nitrogen content of the different-aged portions of the trees throughout the season expressed as: *A*, Percentage of the total; *B*, grams per portion; *C*, percentage of dry matter.

at the beginning of the season, and nitrogen seemed to move out of the roots and into the upper portion of the tree more rapidly during May.   During the latter part of June the roots increased in nitrogen as well as in potassium and the increase continued generally throughout the remainder of the sampling period.

The data calculated on an absolute basis are shown in figure 9, *B*. Here the results closely resemble the potassium data, the only conspicuous differences being that there was a low point in the nitrogen content during June and the nitrogen content of the root fraction increased greatly after November 3.   In the case of potassium, the
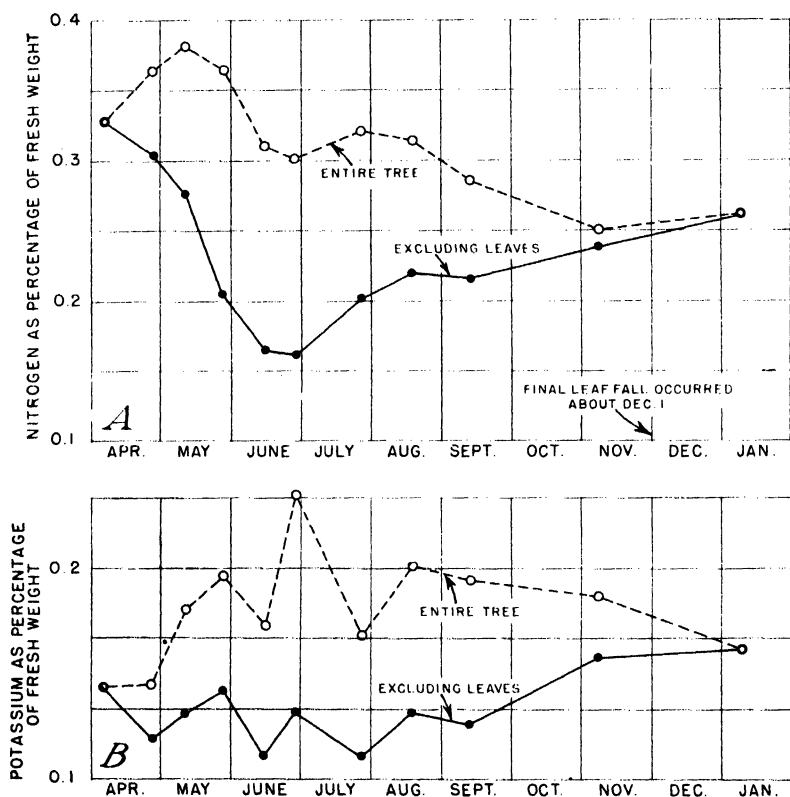
FIGURE 10.   Nitrogen (*A*) and potassium (*B*) content of the trees as a whole throughout the season, both including and excluding the leaves.   Final leaf fall occurred about December 1.

increase on an absolute basis was rapid in all fractions during June and there was no increase in the roots after November 3.

The data on a percentage dry-weight basis are shown in figure 9, *C*. The curves for nitrogen and potassium again are similar, except that the roots decreased greatly in nitrogen during May and early June, and increased after November 3.   Furthermore, the concentration of nitrogen in the 1932 growth dropped off during May much more rapidly than did the potassium concentration.

When the same data were calculated for the bark and wood separately, it appeared that the decrease in nitrogen on a percentage

basis in the 1933 wood was caused largely by changes in the wood
rather than in the bark.   In the lower portions of the tree the nitrogen
changes in bark and wood were rather highly correlated.

Lincoln and Bennett (*12*) report that with pear trees the nitrogen
content on a percentage fresh-weight basis remains rather constant
for the entire tree throughout the season, but when the leaves are
excluded from the calculation, the percentage composition drops to
low values during the middle of the summer.   In view of these re-
sults, it is interesting to compare similar data with apple trees, and
also to see whether potassium presents a similar picture.

Figure 10 shows the percentage of fresh weight for the nitrogen
and potassium content of the tree throughout the season.

In general the principle reported by Lincoln and Bennett held in
the case of nitrogen (fig. 10, *A*).   However, the percentage com-
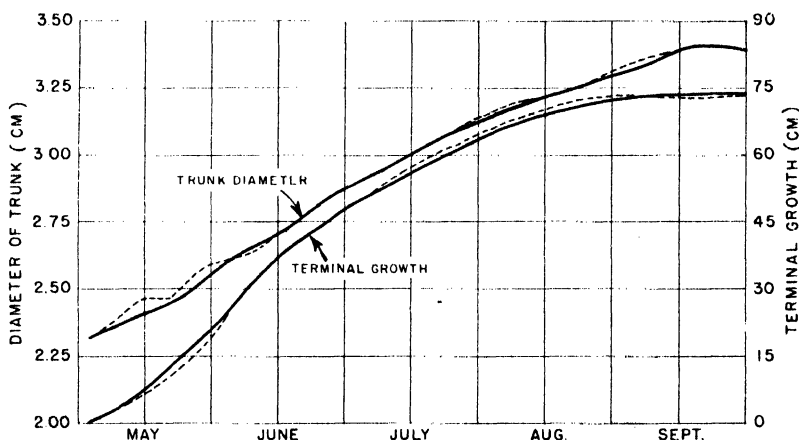
FIGURE 11. Seasonal growth curves of trees treated with potassium sulphate as compared with those of
trees denied potassium. Dotted lines indicate growth of potassium-treated trees when other than
normal.

position of the tree as a whole increased during the early part of the
season and decreased generally from May to November.   In other
words, the increase in nitrogen during April and early May was
greater than the increase in fresh weight, whereas during the summer
the reverse was true.   During the winter, after weight increase had
ceased, nitrogen absorption must be assumed to account for the
increased percentage composition.

The potassium percentages did not act similarly (fig. 10, *B*); the
potassium content, exclusive of that in the leaves, tended to remain
somewhat more constant throughout the season, while in the tree
as a whole it increased during the summer.   In comparison, nitrogen
moved into the leaves without being replaced in the tree proper
until later in the season, while potassium was replaced nearly suffi-
ciently in the bark and wood to make up for any potassium that
moved into the leaves.

GROWTH RESPONSES

Figure 11 shows the growth curves of the trunks and terminals
of the trees.   It is evident that potassium fertilizer had no appreciable
effect on the growth of the trees.   This conformity of growth under

the two treatments is somewhat surprising since the treated trees contained so much more potassium as well as more nitrogen than the untreated ones.

DISCUSSION OF RESULTS

In the light of the findings of this study, it may be stated that the absorption of potassium by apple trees is proportional to the accumulation of dry weight. Under the high potassium treatment, potassium was absorbed at a greater proportionate rate than dry weight was accumulated. Apparently potassium was absorbed largely during the growing period, although it is certainly abundant in the soil solution during the winter. The trees apparently were able to absorb nitrogen after the first part of November, indicating that the tree is able to absorb certain nutrients even when its metabolic rate is considered to be rather low.

One of the outstanding tissue relationships noted in connection with potassium movement was the marked increase in the potassium content of the roots during the latter part of the growing season. The causal factors involved are not understood. Butler and his associates (5) have reported an increase in starch and sucrose in the roots toward the close of the growing season, and if potassium metabolism is similar to that of carbohydrates there is a possible utilization of additional potassium in the roots at this time, perhaps in the role of aiding translocation, as suggested by some workers. Furthermore, the same potassium might function anew in the spring when the stored carbohydrates were broken down and translocated to more active parts of the tree.

This investigation has substantiated the conclusion, already reached with other plants, that potassium is definitely associated with meristematic tissue, is very mobile, and seems to accumulate in certain tissues toward the close of the growing season.

The fact that an extremely large proportion of potassium is located in the leaves is interesting and may add to the importance of leaf-area relationships in fruit-tree responses. The fact that the leaves on the trees from the potassium-treated plots abscissed earlier in the fall may indicate that any excess of potassium is translocated to the leaves for disposal, thus partially accounting for the large luxury consumption in the leaves.

The decreasing concentration gradient of both nitrogen and potassium from the apex to the base of the tree is most logically accounted for by the decrease in the proportion of living to dead cells. The absolute-amount gradient is in the opposite direction, due to the fact that the dry weight of the fractions decreased markedly from the base to the top of the tree.

The relation of potassium to nitrogen seems to be largely a common cause association; that is, nitrogen and potassium both being essential to the life of the cell, more nitrogen and potassium occur where more living cells exist. However, the removal of nitrogen from the bark and wood of the tree as leaf formation takes place, is very marked, whereas no such movement occurs with potassium. Apparently potassium is absorbed from the soil rapidly enough to maintain a supply in the bark and wood and still supply the leaves with adequate amounts.

The increased nitrogen content of the potassium-fertilized trees cannot be adequately explained. If more growth had been produced by the fertilized trees, possibly it would account for the phenomenon. However, the fact that the greatest increase in nitrogen occurred after growth had slowed down would seem to reduce the possibility of any growth response resulting from the nitrogen content. The fact that the increased nitrogen was largely in the wood and root bark might suggest a relationship between potassium and the storage of nitrogen. Any increased supply of nitrogen in a plant, without growth correlation, would possibly result in more reserve nitrogen. Colby (*6*) showed that a potassium deficiency reduced nitrate absorption, but his finding is hardly applicable in the present case since no symptoms of potassium deficiency were observed.

The increased absorption of nitrogen subsequent to leaf fall is in keeping with the findings of Bauer (*3*) in his work with the horse-chestnut and of Combes (*7*) with the beech tree. However, other forest-tree species show other periods of nitrogen absorption. Aldrich (*1*) and Sullivan and Kraybill (*17*), working with the apple, and Schrader (*15*), with the grape, noted marked absorption of nitrogen in the fall.

Relative to the lack of growth response to the applied potassium, the fact should be stressed that if sufficient potassium accumulated in the leaves under the heavy potassium application to cause early abscission, it might have been sufficient to exercise a detrimental effect on growth and thus offset any beneficial effect of potassium fertilizers. On the other hand, the accumulation of nitrogen or potassium in one growing season might have a beneficial effect on growth the following year.

SUMMARY AND CONCLUSIONS

The absorption of potassium by young apple trees was proportional to dry-weight accumulation; it started slowly, continued at a rather rapid rate during the growing season, and then slowed down as cessation of growth approached in the fall.

The trends for the relative amounts of potassium showed that the new growth generally increased throughout the season, while the "1-year" and "2-year" tissues decreased in their relative proportions.

The amount of potassium in the roots seemed to decrease markedly during the time of rapid growth, but increased during the latter part of the season.

The absolute amount of potassium in all portions of the tree generally increased throughout the season. This was not true of the leaves, however, after abscission began.

On a percentage dry-weight basis the potassium content of the current season's growth decreased throughout the season. The roots tended to increase in potassium concentration during the last few months of growth. The 1-year growth increased in percentage of potassium during May but later decreased. The 2-year growth remained constant in its potassium concentration.

The new wood added by diameter growth corresponded closely to the current season's twig growth with respect to potassium concentration.

Young leaves contained much more potassium than old leaves. This difference may have been due either to leaching or to migration.

Generally the concentration of potassium decreased from the apex to the base of the tree, while in actual amount present the reverse was true.

The application of potassium to the soil caused an increased intake of potassium by the tree, and an increased concentration in all parts. The greatest increase was in the leaves, the next in the bark, and the lowest in the wood.

Heavy applications of potassium increased the nitrogen content of the trees.

Nitrogen and potassium were rather highly correlated in the trees. The most noticeable differences were that nitrogen was absorbed late in the season while potassium absorption stopped at leaf fall, and the nitrogen content of the tree was depleted considerably during the summer by the movement of nitrogen into the leaves. With potassium, absorption from the soil was able to maintain a uniform concentration in the wood and bark, and still maintain the content of the leaves.

There were no growth differences among the trees which could be attributed to the influence of potassium fertilizer.

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DISTRIBUTION OF TOTAL SOLUBLE SOLIDS AND CATA-LASE IN DIFFERENT PARTS OF JONATHAN APPLES[1]

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INTRODUCTION

The purpose of the present paper is to present the results of some studies on the variation of concentration and distribution of total soluble solids and catalase in different parts of normal and soft-scalded Jonathan apples (*Malus sylvestris* Mill.). It is not the aim or purpose to attempt any correlations or relationship with soft scald of apples, as the investigation is still in progress and the data presented herein are only preliminary in nature. The data show a rather wide variation in total soluble solids and particularly in the catalase activity between affected and sound apples and between different parts of the same apple. Other chemical fractions would probably show similar variations. That different sections of tissue, although anatomically similar, vary in certain chemical constituents and enzymic activity is emphasized.

REVIEW OF LITERATURE

A number of workers have noted wide differences in chemical constituents in different parts of horticultural fruits and in different plant materials (*7, 8, 11, 12*).[2]

Lall (*11*) found that halves of the same apple varied in sugar content, although this variation was considerably less than that between different apples. Estimates of the sugar content of different parts of Bramley Seedling apples were made by Haynes and Archbold (*9*) and Archbold and Barter (*1*) with the object of determining the most satisfactory method of cutting, so far as symmetrical distribution of sugar was concerned. Haynes and Archbold show that sugar concentration increases from the stalk (stem) end toward the calyx, from the inside toward the outside, and is lower on the unblushed than on the blushed side of the fruit.

Fisher, Harley, and Brooks (*3*), working with water core of apples, found that in its early development, when the affected tissues were very localized, these parts were characterized by rapid starch conversion with a corresponding increase in soluble sugars as compared with adjacent unaffected tissues. These workers also found a very pronounced decrease in titratable acidity associated with the increase of moisture and soluble sugars in water-cored apples.

Miller (*13*) determined acetaldehyde and ethyl alcohol on duplicate samples of Jonathan apples, and found variations in the amount of these constituents in the different parts of the fruit. There was an apparent accumulation of acetaldehyde and alcohol in the peel as compared with the pulp and cores, and the concentration was much higher in the peel of fruits with soft scald than in the normal peel.

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[1] Received for publication Dec. 6, 1935; issued August 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 47.

METHODS

The apples for the present investigation were all of the Jonathan variety and were obtained from orchards at Hancock, Md. The fruit was picked September 12, 1935, trucked the same day to the cold-storage laboratory at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.), and held under temperature conditions conducive to the development of soft scald. These conditions consisted of 6 days' delayed storage at 65° F., followed by transfer of the fruit to 32° for the remainder of the 90-day holding test. This particular study was a part of a general investigation on soft scald of apples.

The method employed for taking the pulp sample was similar to that reported by Carrick (*2*) and Harding (*5*). A cylinder of tissue was taken through the transverse center of 25 to 30 apples by means of a cork borer. From this tissue, sections representing various locations (fig. 1) were cut and composited. Because of the variation in ground color of the fruit, the skin sample was obtained by peeling very thin a narrow section around the greatest transverse diameter of the apple.

Total soluble solids were determined by refractometer readings made on the juice extracted from each sample after grinding in a mortar and straining through a double thickness of muslin. The usual precautions for accurate refractometer determinations were taken, and corrections were made for temperature variations. Duplicate samples of juice were prepared from each lot of fruit and two refractometer readings were made on each sample. These readings in every case checked within two points in the fourth decimal place.
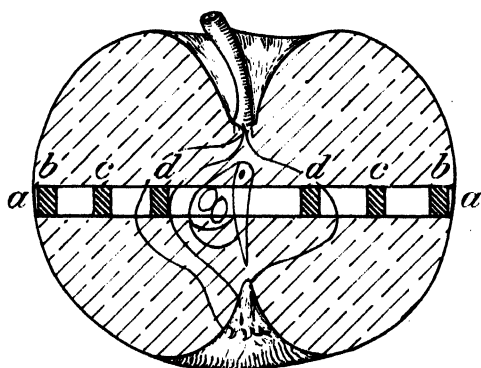
FIGURE 1.—Section of apple showing location of cylinder removed for sampling. Skin and hatched portions of cylinder represent material used for analysis: *a*, Skin; *b*, one-sixteenth inch beneath skin; *c*, halfway between skin and core; *d*, pith region.

For catalase determinations, 4 g of tissue (skin or pulp) was weighed immediately and mixed with an equal weight of calcium carbonate and 2 ml of distilled water. An excess of calcium carbonate was used to neutralize the juice. To eliminate any differences in pH, which might possibly cause variations in catalase activity, the same amount of calcium carbonate was used in all catalase determinations. The sample was ground in a mortar until a creamy mixture was obtained. In the case of the skin sample, a small amount of clean quartz sand was added to facilitate grinding. The suspension was brought to a volume of 25 ml, with the exception of the samples of the skin and pith region, where greater dilutions were necessary in order to make readings on the measuring and leveling burettes having a capacity of 50 ml. Where the greater dilutions were made, the final catalase reading was calculated so as to be representative of the same weight of tissue.

Two samples of Komer's Antegin (catalase activator) were obtained from the Bureau of Chemistry and Soils, United States Department of Agriculture, and tested with apple tissue. When used at dilutions of 1 to 100, 1 to 500, and 1 to 1,000, there was little or no increase in catalase activity.

Qualitative tests for peroxidase determinations were made and positive results were obtained. It was difficult to evaluate the amount of the enzyme present in the skin region. The pith and regions surrounding the core indicated high peroxidase activity. The same region was also high in catalase activity. Undoubtedly peroxidase affected the results obtained for catalase. However, the manometer method for catalase determination is one of the standard methods and the error from the decomposition of hydrogen peroxide by peroxidase is believed to have been a constant error.

Standard procedure for determining catalase activity was followed, similar to that used by Haber (*4*) and Harding (*5*). Five milliliters of the skin or pulp-tissue suspension was placed in one arm of the catalase tube and 5 ml of hydrogen peroxide was placed in the other. The tube was suspended in a water bath held at a uniform temperature of 70° F., and the solutions in the tube were allowed to reach the temperature of the water bath. Figure 2 shows details of the apparatus. The samples were shaken 15 minutes at a uniform rate of 120 oscillations per minute, after which the amount of evolved oxygen was measured. Every precaution was taken to obtain a uniform sample and a uniform suspension of the macerated tissue. The duplicate samples, or samples from the same part, whether skin or pulp, checked within 0.3 ml of evolved oxygen.

DISTRIBUTION OF TOTAL SOLUBLE SOLIDS

Table 1 shows the amount of total soluble solids as they were found distributed throughout various parts of the apple. It will be noted that the skin tissue contained the largest amount, and that the concentration decreased toward the core region. This is in harmony with the findings of Haynes and Archbold (*9*), who concluded that sugar concentration decreased from the outside toward the inside of the fruit. It might be noted also that samples from normal Jonathan apples had slightly greater amounts of total soluble solids than did comparable samples from apples showing soft scald (table 1).

TABLE 1.—*Concentration and distribution of total soluble solids and catalase activity in normal and soft-scalded Jonathan apples* [1]

Sampling region	Total soluble solids		Catalase activity	
	Normal apples	Soft-scalded apples	Normal apples	Soft-scalded apples
	Percent	*Percent*	*Milliliters* [2]	*Milliliters* [2]
Skin only	15.96	[3] 15.47	95.00	[3] 4.00
¹⁄₁₆ inch beneath skin	13.23	[4] 12.47	11.60	[4] .10
Halfway between skin and core	13.03	[5] 12.97	19.00	[5] 27.60
Pith [6]	12.72	[5] 12.42	34.50	[5] 45.00

[1] 4 pairs of samples, or 8 altogether, consisting of from 25 to 30 apples each, were used. Each of the 8 samples was tested in duplicate, and the final results shown are averages from the 4 analyses of each pair of samples.
[2] Milliliters of oxygen evolved at the end of 15 minutes.
[3] Skin scalded.
[4] Tissue brown.
[5] Tissue normal in appearance.
[6] Kraus (*10*) designates the region between the primary vascular bundles and core as the pith.
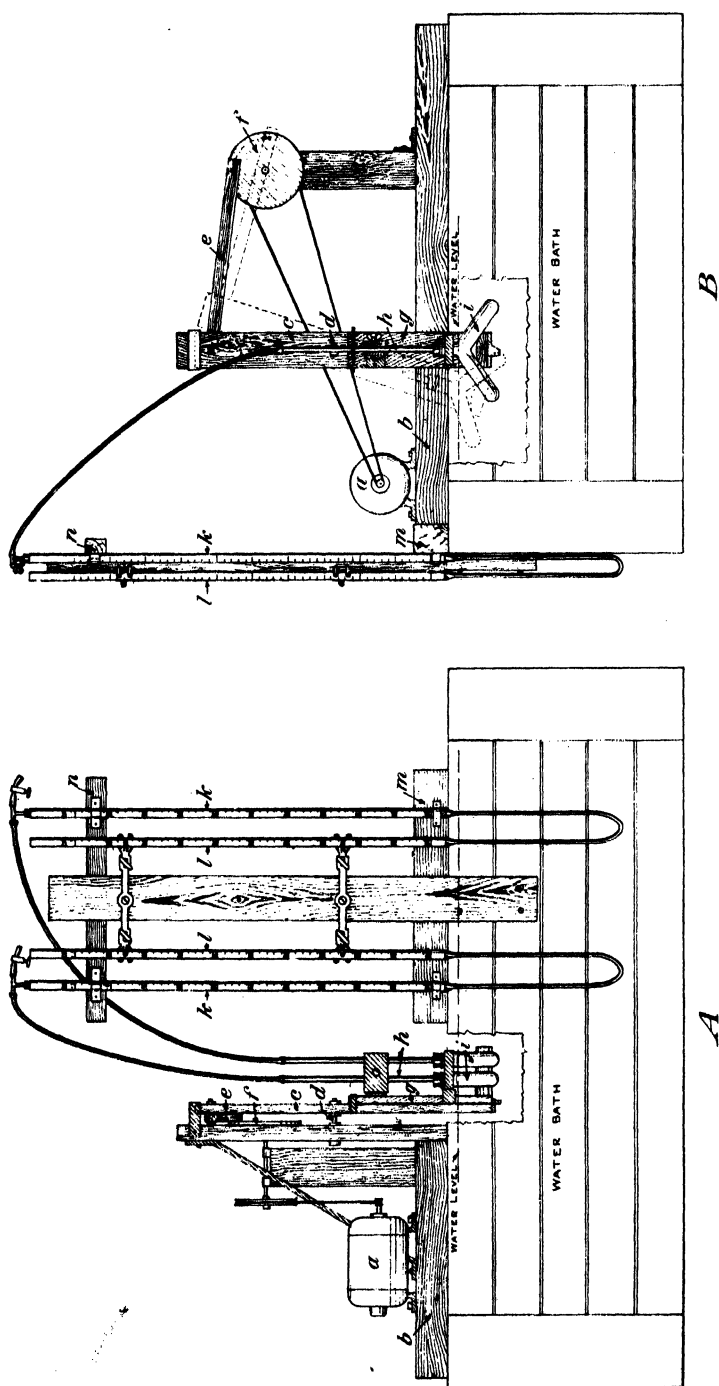
FIGURE 2.—Apparatus for duplicate catalase determinations: 1, Front view; B, side view. a, Motor; b, platform over water bath; c, shaker arm; d, pivot for shaker arm; e, connecting rod; f, driving disk for connecting rod; g, catalase-tube holder; h, connecting glass tube; i, catalase tube; j, leveling burette; k, stationary burette; l, leveling glass tube; m, cleat; n, crosspiece.

DISTRIBUTION OF CATALASE

The data on the distribution of catalase activity are also presented in table 1 for purposes of convenience and without intent to imply any correlation between them and the data for total soluble solids.

In normal apples, catalase activity was highest in the skin and least in the region immediately beneath the skin, increasing toward the core (table 1). In apples showing soft scald, the least catalase activity was noted in the diseased portion comprising the skin and the brown tissue immediately beneath. It seems quite possible that in peeling the scalded skin a small amount of normal skin might have been included in the sample. This would account for the reading of 4 ml of oxygen evolved, since the brown tissue beneath the scalded skin showed only 0.1 ml of evolved oxygen (table 1).

Aside from the skin tissue, the regions of highest catalase activity were the pith and the parenchyma tissue halfway between core and skin (table 1). In these two regions catalase activity was significantly higher in soft-scalded apples than in corresponding samples from normal apples. The writer (6) has observed that high catalase activity was registered just prior to the appearance of soggy breakdown in Grimes Golden apples.

SUMMARY

Specific differences in total soluble solids and catalase activity were found in different parts of Jonathan apples.

Soluble solids were consistently lower in apples affected with soft scald than in those not affected, but in both cases the greatest concentration was found in the skin, with a gradual decrease toward the pith.

In normal apples, catalase activity was highest in the skin and least in the region immediately beneath the skin. In apples showing soft scald, catalase activity was highest in the pith region and lowest in the diseased portion comprising the skin and the brown tissue immediately beneath it.

The results of this investigation suggest that preliminary work to determine localized differences in different parts of an apple should precede chemical or physiological studies in which the fruit is customarily analyzed as a whole. This procedure might lead to a modification of methods of sampling and give quite different and more significant results.

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DISTRIBUTION OF ACETALDEHYDE AND ALCOHOL IN THE APPLE FRUIT [1]

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INTRODUCTION

In the course of an investigation, which is still in progress, concerning the role of acetaldehyde in the metabolism of stored fruit, several interesting observations were made regarding the distribution of acetaldehyde in the apple fruit. Studies of the distribution of soluble solids and catalase activity were conducted on the same lot of fruit by Harding (5).[2] The present paper reports the data concerning the distribution of acetaldehyde and alcohol in the fruit.

REVIEW OF LITERATURE

It has long been known that acetaldehyde and alcohol may appear as products of metabolism in many higher plants stored under anaerobic conditions. Thomas (10) has reviewed the earlier work rather thoroughly, and reference is made here only to recent literature that pertains to the distribution of these substances in the fruit. Power and Chesnut (9), in their work on the odorous constituents of apples, stated that these substances appeared to be contained chiefly in the outer skin or rind of the fruit, and their analyses of the apple parings indicated the presence of several different esters as well as acetaldehyde.

Gerhardt [3] has shown that the peel of Jonathan apples is higher in acetaldehyde than the pulp of either normal apples or of those showing soft scald; furthermore, that the acetaldehyde content of peel from the diseased apples is higher than that of normal apples.

Kidd and West (7) exposed apples to ethyl alcohol vapor and analyzed the cores and "inner" and "outer" sections of the pulp for acetaldehyde and alcohol. They reported that while the alcohol is being taken up the largest amount of ethyl alcohol is found in the cores and that while the alcohol is escaping the reverse is true. The aldehyde content during alcohol absorption was highest in the "outers", but during alcohol escape there was little difference in the acetaldehyde content of these three portions of the apple.

Fisher, Harley, and Brooks (3) reported that appreciable quantities of ethyl alcohol are present in the water-cored area of apple fruits and that under certain conditions relatively large quantities may be found.

MATERIAL AND METHODS

The Jonathan apples used in these experiments were obtained from the American Fruit Growers' orchard at Hancock, Md. They were picked September 11, 1934, delayed 6 to 10 days at 18° C., and then stored at 0° until December 11, 1934, when they were analyzed.

The Grimes Golden apples used for the study of soggy break-down were also grown at Hancock and received the same storage treatment except that they were held in storage till February 11, 1935. The Grimes Golden apples used in the experiments on freezing and those used in comparing the different methods for preparing pulp samples were grown at Vienna, Va., about 10 miles from Washington, D. C. These apples were harvested October 9, 1935, and were subjected to the experimental tests immediately after harvesting.

Thirty Jonathan apples were employed for each sample. The apples were separated into three parts—peel, pulp, and cores. The peel was removed by a mechanical peeler and the cores were cut out with a 2-cm cork borer. The peeled and cored apples were then cut into slices small enough to slip through the neck of an 800-ml Kjeldahl flask. This constituted the pulp sample.

One-hundred-gram duplicate samples were analyzed for acetaldehyde by the method described by Harley and Fisher (*6*). This consists of steam-distilling the samples for 90 minutes and collecting the distillate in a standard solution of sodium bisulphite. Inasmuch as this is a rather delicate determination, extreme precautions were observed in order to reduce the chances of error. The solutions of iodine and sodium bisulphite were standardized each day and stored in the refrigerator when not in use. The sodium bisulphite was delivered from a burette stoppered with a soda-lime tube and was not added to the receiving flasks until they had been cooled in the ice bath and were ready for immediate connection with the condensers. Instead of titrating the bisulphite with iodine, a known quantity of iodine solution was added to the distillate and this was titrated "back" with sodium thiosulphate. With these precautions, in addition to those described by Harley and Fisher, duplicate distillations were obtained which checked within 0.1 or 0.2 ml of N/10 $Na_2S_2O_3$.

Alcohol determinations were made by the method described by Thomas (*10*). This consists of removing the acetaldehyde and oxidizing the alcohol to acetic acid.

RESULTS OF ANALYSES

JONATHAN APPLES

Table 1 shows the quantities of acetaldehyde and ethyl alcohol found in the different parts of Jonathan apples. Both sound fruit and similar apples affected with soft scald were studied. There was an apparent accumulation of acetaldehyde and alcohol in the peel as compared with the pulp and cores, and the concentration was much higher in the diseased than in the normal peel.

TABLE 1.—*Acetaldehyde and ethyl alcohol content of peel, pulp, and cores of Jonathan apples*

Tissue analyzed	Acetaldehyde in—			Alcohol in—		
	Peel	Pulp	Cores	Peel	Pulp	Cores
	Mg^1	Mg^1	Mg^1	Mg^1	Mg^1	Mg^1
Normal	1.0	0.0	0.3	28.8	17.0	11.6
Soft scald	4.1	.5	.5	36.0	11.6	12.6

[1] Per 100 g of fresh tissue.

An additional lot of Jonathan apples was analyzed in which the pulp was separated into inner and outer portions. In this lot (table 2) also the peel showed the highest concentration of acetaldehyde. Each portion of the diseased apples showed a higher concentration than did the corresponding portion of the normal apples. The inner pulp was usually higher in acetaldehyde than the outer pulp.

An attempt was made to analyze the cuticle of the apples after removal by the method recommended by Markley and Sando (8), but it was impossible to prepare the material for analysis without introducing errors. Instead, the apples were immersed quickly in ether, rinsed in water, and dried. The peel was then analyzed for acetaldehyde and total ether extract in order to ascertain whether the removal of the ether-soluble fraction of the cuticle removed much of the acetaldehyde. From table 3 it will be noted that the rinsing in ether removed 16.3 percent of this ether-soluble fraction and 36.7 percent of the acetaldehyde.

GRIMES GOLDEN APPLES

The studies were later extended to include Grimes Golden apples showing soggy break-down. This disease is characterized by a browning of the pulp, whereas soft scald is limited to the peel and a small portion of the pulp directly beneath it. In these analyses (table 4) the peel again showed higher acetaldehyde than the pulp or cores and the peel from the diseased apples showed higher acetaldehyde content than that from the normal ones. The aldehyde content of the cores was higher in the diseased than in normal apples; the reverse was true of the pulp.

TABLE 2.—*Acetaldehyde content of peel, outer pulp, inner pulp, and cores of Jonathan apples*

Tissue analyzed	Peel	Outer pulp	Inner pulp	Cores
	Mg^1	Mg^1	Mg^1	Mg^1
Normal	2.0	0.3	0.65	0.45
Soft scald	6.0	1.7	2.3	.55

[1] Per 100 g of fresh tissue.

TABLE 3.—*Acetaldehyde and ether extract removed from peel by immersing Jonathan apples in ether*

Tissue analyzed	Acetalde- hyde	Ether extract
	Mg [1]	*Mg* [1]
Peel	4.9	0.190
Peel washed with ether	3.1	.159
	Percent	*Percent*
Loss	36.7	16.3

[1] Per 100 g of fresh tissue.

TABLE 4.—*Acetaldehyde content of peel, pulp, and cores of Grimes Golden apples*

Tissue analyzed	Peel	Pulp	Cores
	Mg [1]	*Mg* [1]	*Mg* [1]
Normal	6.2	2.8	0.0
Soggy break-down	9.1	2.4	1.9

[1] Per 100 g of fresh tissue.

It was found that comparable duplicate results could not be obtained unless the analyses were made simultaneously. Fidler (*2*) states that sliced apples exposed to air for a half hour may lose significant amounts of acetaldehyde. In the present work the discrepancies were apparently due to accumulation rather than to loss of acetaldehyde. Samples of peel, pulp, and cores of Grimes Golden apples were weighed, transferred to 800-ml Kjeldahl flasks, stoppered, and held in the refrigerator. Acetaldehyde determinations made at the end of 1, 2, and 4 hours showed definite increases during this time (table 5). At the end of 4 hours the acetaldehyde content of the peel and pulp had approximately doubled; that in the cores had quadrupled.

TABLE 5.—*Accumulation of acetaldehyde in cut apple tissue analyzed immediately and after storage for 1, 2, and 4 hours in a flask in the refrigerator*

Time of sampling	Peel	Pulp	Cores
	Mg [1]	*Mg* [1]	*Mg* [1]
Immediately	6.8	1.1	0.9
After 1 hour		1.8	
After 2 hours	13.1	1.6	
After 4 hours	13.4	2.6	3.7

[1] Per 100 g of fresh tissue.

It has been suggested that the high acetaldehyde content of the peel in these experiments may be traceable to its rapid formation in the cells injured through peeling the apples, rather than to its adsorption by the peel. A further study of this phase was made by comparing finely sliced pulp samples with those of the peel. Thirty Grimes Golden apples were treated with carbon dioxide to increase the acetaldehyde content. The peel was removed by the mechanical peeler, and the apples were then replaced on the machine and strips of pulp were removed in the same manner as the peel. The results of the acetaldehyde determinations appear in the first line in table 6.

TABLE 6.— *Content and distribution of acetaldehyde in Grimes Golden apples under various conditions*

Treatment	Total acetalde-hyde content	Acetaldehyde distribution			
		Peel	Pulp		
			Thin sections	Sliced	Finely ground
	Mg^1	Mg^1	Mg^1	Mg^1	Mg^1
Treated with CO $_2$		2.2	1.5		
Normal		.6		0.0	0.4
Do	0.1				
Frozen [2]	.1				
Normal [3]	.3				
Frozen [4]	1.1				

[1] Per 100 g of fresh tissue.
[2] 18 hours in freezing room; 4 hours in laboratory.
[3] Held for 24 hours in the laboratory before being analyzed.
[4] 18 hours in freezing room; 24 hours in laboratory.

This experiment was repeated with normal Grimes Golden apples. In this instance the pulp was prepared in two ways. After the apples had been peeled and cored, one half of each of the 30 apples was cut in slices small enough to pass through the neck of an 800-ml Kjeldahl flask; the other half was ground in a food chopper. It is probable that the high acetaldehyde content of the peel, as shown in table 6, may be due both to the large number of cells injured in the peeling process and to the tendency of the acetaldehyde to accumulate in the peel.

Another question that arises is whether apple cells injured by freezing produce acetaldehyde in abnormal quantities. This was investigated in the following manner. Thirty Grimes Golden apples were held in the freezing room for 18 hours. The temperature of the room was about —8° C. The temperature of the apples when removed was —3.5° at the core. They were definitely frozen but not permanently injured. When the apples were thawed the flavor was not quite so good as that of the controls, but they would no doubt have been readily accepted by the average consumer. They were held in the laboratory for 4 hours and then analyzed for acetaldehyde. Samples consisted of the entire edible portion of the apple. Controls were held in the cold-storage room at 0° during the time the others were being frozen and were then transferred to the laboratory along with the frozen lot.

This experiment was repeated with the modification that the apples were held in the laboratory 24 hours before analyzing for acetaldehyde. It will be noted (table 6) that in the first experiment the frozen apples produced no more acetaldehyde than did the normal apples. In the second experiment, however, when the apples were held in the laboratory 24 hours following freezing, the acetaldehyde content was more than three times as great in the frozen as in the normal apples.

DISCUSSION

Other investigators have reported that the outer portion of the apple fruit is more active physiologically than the region near the core. Fellers, Isham, and Smith (*1*) have shown that the vitamin C

content of Baldwin and McIntosh apples is concentrated in the epidermis and fleshy cortex. In the Baldwin variety the epidermis was about 4 times as active in vitamin C as the flesh near it, and 6 to 10 times as active as the flesh in the pulpy area near the core. Harding (5) found that in normal Jonathan apples the skin showed both higher catalase activity and higher soluble solids than the pulp at different depths between the peel and core.

In earlier stages of the present investigation it appeared that acetaldehyde was produced most extensively in that portion of the apple that is removed by a mechanical peeler. Subsequent experiments, however, suggested that the amount of acetaldehyde formed may depend upon the number of cells ruptured. It is also possible that the acetaldehyde accumulates in the cuticle of the apple as suggested by Gane (4). It is significant that the peel of apples affected with either soft scald or soggy break-down always showed higher acetaldehyde content than that of normal apples, and this was true whether the disease was manifested in the peel or in the pulp of the apples. It is apparent, then, that whether the apple is producing acetaldehyde as a result of physiological disorder or of mechanical injury the acetaldehyde tends to accumulate in the peel.

SUMMARY

The acetaldehyde content and the alcohol content of Jonathan apples have been shown to be higher in the peel than in the pulp or cores, and higher in the peel of soft-scalded Jonathan apples than in that of normal apples.

Rinsing Jonathan apples in ether removed 16.3 percent of the total ether extract of the peel. As a result of this process the peel lost also 36.7 percent of its original acetaldehyde content.

The acetaldehyde content of normal Grimes Golden apples is higher in the peel than in the pulp or cores. Acetaldehyde content is higher in the peel of Grimes Golden apples showing soggy break-down than in that of normal apples.

Samples of peel, pulp, and cores prepared for analyses but held in stoppered flasks in the refrigerator 4 hours accumulated from two to four times the original amount of acetaldehyde.

Finely ground pulp of normal Grimes Golden apples yielded more acetaldehyde than coarsely sliced pulp but not so much as the peel.

Frozen Grimes Golden apples when sampled 4 hours after freezing showed no more acetaldehyde than normal apples. When held 24 hours in the laboratory following freezing the acetaldehyde content was more than three times as great in the frozen apples as in the normal apples.

It appears that high acetaldehyde content of apple peel may be due in part to its production by cells ruptured in the paring process, but there is also a tendency for the acetaldehyde to accumulate in peel affected either by mechanical injury to the cells or by abnormal physiological conditions of the fruit.

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THE ROLE OF THE CAPILLARY POTENTIAL IN THE DYNAMICS OF SOIL MOISTURE [1]

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For the movement of water horizontally it is natural to assume that the greater the pressure difference between neighboring points the greater will be the velocity. Darcy discovered a relation which is expressed in the following simple form:

$$v = \frac{k(p_1 - p_2)}{l} \tag{1}$$

where v is the velocity of water flowing through a horizontal pipe containing sand, p_1 the pressure at one end and p_2 the pressure at the other end, l the length of the tube, and k a constant of proportionality. This law has been observed by several investigators, but its far-reaching significance has not been widely known or understood.

It is apparent that for vertical flow the equation is inadequate. The factor $\frac{p_1 - p_2}{l}$ represents in this special case a vector quantity, which has been called the gradient of the pressure p. It is the force per unit volume due to variation in pressure from point to point. It may be expressed by the concise symbol ∇p. Dividing ∇p by the density ρ of the fluid reduces it to a force per unit mass. Conservative forces (forces which do not arise from friction) are frequently expressed as a matter of convenience as the gradients of potential functions. Thus, the gravitational field intensity g is set equal to the negative gradient of the gravitational potential function ϕ

$$g = -\nabla\phi \tag{2}$$

In the case of horizontal flow the direct effect of gravity is balanced out in such a way as to have no influence on the motion, and for this reason does not appear in equation (1). For the general case it is necessary to modify the equation thus

$$v = -k\rho((1/\rho)\nabla p + \nabla\phi) \tag{3}$$

For velocities which are reasonably small this equation represents a successful dynamical background for the movement of water in saturated soils.

Conciseness may be gained by introducing a new symbol for the first term in the parenthesis of the right-hand side of equation (3). If we take into account the slight compressibility of water, and

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therefore the slight variation in ρ, a function ψ of the position coordinates, x, y, z, satisfying the following vector equation

$$\nabla \psi = (1/\rho) \nabla p \tag{4}$$

may be found, provided only that ρ is a function of p. Except for an arbitrary integration constant this function differs only slightly from the ratio p/ρ, and it is to this function that the name capillary potential has been given.

If we introduce the symbol Φ, defined thus

$$\Phi = \psi + \phi \tag{5}$$

it is possible to write the generalized Darcy equation in the concise form

$$v = -k\rho\nabla\Phi \tag{6}$$

Whether or not the concise symbolism is to be desired, it still remains a fact that the physical quantity ψ, which we call the capillary potential, plays a significant role in this important basic equation.

This method of attack has suppressed the fact that the actual region occupied by water in the soil is bounded by a much distorted and complicated solid-water interface. For unsaturated soils a new bounding air-water interface appears, and if we confine our attention to the water region thus bounded we are still confronted with the physical concepts of pressure, density, capillary potential, etc., and they have the same meaning except that the pressure is throughout the region less than atmospheric and it might more appropriately be designated as capillary tension. The term "capillary potential" was introduced first by Buckingham[2] in a discussion of moisture phenomena in unsaturated soils, and in the literature published at the Utah Agricultural Experiment Station its connection with unsaturated soils has been stressed. Although defined with reference to this water phase it also will be construed in a macroscopic sense as a point-characteristic of the soil.

To measure it a porous cup filled with water is brought into close contact with the soil and the tension in the soil water is transmitted to the water in the cup and thence to an ordinary manometer. For soils sufficiently dry to have a "tension" as great as an atmosphere this method is inadequate.

It is interesting to observe, however, that there is a close connection between the capillary potential and the corresponding vapor pressure. If water is permitted to ascend a column of soil from a free-water surface it is evident that at each point above the free water when equilibrium is established a definite moisture percentage, a definite capillary potential, and a definite vapor pressure will be established. The capillary tension at a height h from free water would be $\rho g h$, whereas the vapor pressure π would decrease exponentially from a value π_0, thus,

$$\pi = \pi_0 e^{-kh} \tag{7}$$

or, in another form,

$$h = -\frac{\log_e (\pi/\pi_0)}{k} \tag{8}$$

[2] BUCKINGHAM, E. STUDIES ON THE MOVEMENT OF SOIL MOISTURE. U. S. Dept. Agr. Bur. Soils Bull. 38, 61 pp., illus. 1907.

leading to the relation

$$\psi = -\frac{g \, \log_e \, (\pi/\pi_0)}{k} \tag{9}$$

It seems evident a priori that the constant factor $k\rho$ can no longer completely represent the so-called transmission constant, but that some as yet undetermined function $f_1(\rho')$ of the variable moisture content ρ' of the soil, involving parameters characteristic of the soil, should be substituted. Furthermore, although, as stated, there is no essential change in the connection between the capillary potential and the pressure and density of the water, its actual magnitude is influenced by the amount of moisture in the soil. It will be appropriate, therefore, to introduce for the capillary potential another function $f_2(\rho')$, this function also containing characteristics oil parameters. The Darcy law may thus be generalized to apply to unsaturated soils as follows:

$$v = f_1(\rho')\nabla(f_2(\rho') + \phi) \tag{10}$$

In addition to the Darcy velocity law we have another differential equation which comes directly from the law of conservation of matter and which is called the equation of continuity. To express this equation in concise form a new symbol $\nabla\cdot$, known as the divergence operator, is introduced, thus,

$$\nabla\cdot(\rho'v) = -\partial\rho'/\partial t) \tag{11}$$

This equation means that the divergence of the flux density is equal to the negative time rate of change of the density. Eliminating v from this equation by means of equation (10), we obtain

$$\rho'f_1\nabla^2f_2 + (\nabla(\rho'f_1))\cdot(\nabla f_2 + \nabla\phi) = -\partial\rho'/\partial t \tag{12}$$

A quantity $\nabla^2\phi$, which vanishes on account of the constancy of $\nabla\phi$, has been omitted from the equation. The dot between the parentheses of the second term indicates that the scalar product of the two vectors is to be taken.

New experimental work is required to determine the functions f_1 and f_2. On the basis of the original Darcy law they are known of course for saturated soils, and for the case of steady flow equation (12) reduces to the well-known Laplace equation and it forms the basis for the solution of practical problems concerning the movement of ground water.

Tentative approximations also have been presented for the case of unsaturated soils. The physics laboratory at the Utah station has made use of the following simple substitutions

$$f_1 = k\rho' \tag{13}$$

and

$$f_2 = c/\rho' + b \tag{14}$$

where k, c, and b are empirical constants. Experimental results published heretofore indicate that these represent good first approximations.

Much has been written in texts on applied mathematics concerning the solution of the Laplace equation for various boundary conditions.

A more detailed discussion of the ground-water problem, together with a special application of equation (12) subject to the approximations (13) and (14), has been presented recently.[3]

Various soil characteristics have been defined all of which no doubt serve some useful purpose.   It is to be observed, however, that such characteristics as the transmission factor and the capillary potential enter naturally into the basic analysis of the fundamental dynamical problem of the movement of soil moisture, and it is this problem that is of primary concern to irrigation and drainage engineers.   Their application to the so-called capillary fringe has not been extensive. It is nevertheless significant that capillary phenomena have constituted a nucleus about which has centered much discussion in researches in soil physics since its beginning, and it can scarcely be claimed that the problem has been satisfactorily solved without this or some similar approach from the standpoint of classical dynamics.

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[3] GARDNER, W., COLLIER, T. R., and FARR, D. GROUND-WATER, PART 1. FUNDAMENTAL PRINCIPLES GOVERNING ITS PHYSICAL CONTROL. Utah Agr. Expt. Sta. Bull. 252, 40 pp., illus. 1934.

THE EFFECT OF FERTILIZERS AND LIME UPON THE ELECTRODIALYZABLE AND EXCHANGEABLE POTASH OF CROPPED SOIL.[1]

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INTRODUCTION

The fertility plots maintained at the experimental farm of the Delaware Agriculture Experiment Station offered an opportunity for investigating the effects of fertilizers, manure, and lime upon the electrodialyzable and exchangeable potash of plot soils. The plots were established in 1908 and have been subjected to a definite cropping system and fertilizer program.

REVIEW OF LITERATURE

Humfeld (8) [2] found that fertilizer treatments continued over a term of years have a measurable effect upon the kind and amounts of bases and acids extracted from the soil by electrodialysis.

Holmes (7) and Schollenberger and Dreibelbis (14) found that applications of manure resulted in a slight increase in exchangeable potassium, but Merkle (13) noted no increase.

Volk (17) and Bartholomew and Janssen (3) obtained evidence of the fixation of added potash in nonreplaceable form.

Snider (16) found that replaceable potassium was increased by the continued use of potash salts in Illinois plots, and observed a tendency toward lower replaceable potassium values on the heavily limed soils as compared with the lightly limed. Brewer and Rankin (4) also found that liming decreased the amount of potassium removed by electrodialysis and the Neubauer method. Abel and Magistad (1), however, found that on cropped and fallow pineapple soils liming increased the replaceable potassium, and the crops removed more potassium from the limed soils. In soils deficient in potash the crops removed more potassium than was present in replaceable form, yet in some cases the amount of replaceable potassium was greater at the end of the experiment than at the beginning. Their results are in agreement with those of Hoagland and Martin (5, 6).

MacIntire and his coworkers (12, p. 509) concluded from the results of lysimeter studies that:

As a whole, the results established the fact that the liming of rock-derived soils under humid conditions will depress the hydrolytic disintegration of both the original potassium complex and that formed by the fixation of soluble added potassium salts, and further that the protective, or buffering, effect becomes more pronounced with increased concentration of the bicarbonates of calcium and magnesium.

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Jenny and Shade (*9*), however, found that potassium was liberated by the calcium ion in the presence of Cl, SO_4, CO_3, HCO_3, OH, and PO_4 ions. Their investigation did not include a study of the conversion of nonreplaceable to available potassium.

EXPERIMENTAL METHODS

The plots from which the samples [3] were taken are designated as "block B" of the fertility plots at the experimental farm. The soil is classified as Sassafras silt loam.

The treatments to which the plots were subjected over a period of 24 years are indicated in table 1.

TABLE 1.—*Treatment of the block B fertility plots, 1908–32*

[Quantities applied are per acre]

Plot no.[1]	Muriate of potash	Sodium nitrate	Dried blood	Super-phosphate	Farm manure
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Tons*
4	1,335				
7	1,335			3,900	
8	1,335	1,675			
9	1,335	1,599	75	3,900	
10	2,550	3,150	100	7,300	
14				750	65
15					130

[1] Nos. 6, 11, and 16 were check plots.

Each plot (one-tenth of an acre) was divided into two sections, one of which received lime as follows: 1908 and 1916 at the rate of 2,000 pounds per acre; 1912, 1920, and 1924 at the rate of 1,500 pounds per acre. The last application of lime was made in 1924. Both the limed and unlimed sections received the same fertilizer and manure treatments.

The cropping system employed from 1908 to 1912 was corn, oats, wheat, and timothy and clover; since 1912 it has been corn, soybeans, wheat, and timothy and clover.

Samples were taken in the late summer of 1932. Twenty cores (1⅝ by 6⅝ inches) were taken from each section (lime and unlimed) of each plot with the utmost care to insure samples as representative as possible. The soil was thoroughly mixed, air-dried, and again quartered.

One hundred grams of air-dried soil was electrodialized in a three-compartment Mattson cell. Parchment membranes and perforated sheet-platinum electrodes were employed. With this arrangement the electrodes were approximately 2 cm apart. Current was supplied by a motor generator and applied at 110 volts. The soil was subjected to electrodialysis for 1 hour, the dialyzates removed, and electrodialysis continued for three 8-hour periods, making a total of 25 hours for each sample. Potash was determined in the dialyzate.

One hundred grams of air-dried soil was leached with normal ammonium acetate according to the method of Schollenberger and Dreibelbis (*15*) and potash was determined in the leachate.

[3] The soil samples and data in table 1 were furnished by Dr. H. C. Harris, assistant agronomist of the station.

Hydrogen-ion determinations were made on the soils after they had stood for 3 days, with occasional shaking, in 2.5 times their weight of water. The MacInnes-Dole type glass electrode with vacuum-tube potentiometer was employed.

RESULTS

The course of the current during electrodialysis was remarkably uniform throughout the series, rising to a maximum of approximately 300 milliamperes within a few minutes and decreasing gradually as dialysis progressed until it became practically constant at 30 milliamperes.

The results for dialyzable, exchangeable, and total potash and the pH values for the plot soils are presented in table 2.

Without exception, more potash was removed by electrodialysis from the limed soils than from the unlimed. With the exception of plots 8 and 16, in which the values for limed and unlimed sections are identical, this holds true for exchangeable potash. While exchangeable and dialyzable potash are of the same order, exchange potash is of lesser magnitude. Applications of potash are reflected to a greater degree by electrodialysis than by the exchange method. Both exchangeable and dialyzable potash are at a maximum in plot 15, which received manure only. The total potash varies between 39.7 milliequivalents (plot 4) and 35.3 milliequivalents (plots 14 and 15) in the limed series and between 36.1 milliequivalents (plot 16 check) and 32.9 milliequivalents (plot 11 check) in the unlimed series. In general, however, the potash content is quite uniform throughout each series with that of the limed soil exceeding in every case that of the corresponding unlimed soil.

The pH values are rather uniform throughout the respective series (limed and unlimed) with the lowest for plot 4 (KCl not limed) and the highest for the check plots 6 and 11 (limed).

TABLE 2.—*Effect of fertilizers, manure, and lime upon the electrodialyzable and exchangeable potash of sassafras silt loam*

[Quantities per 100 g of soil dried at 110° C. for 18 hours]

Plot no.	Treatment	Limed	pH	K₂O Total	K₂O Electro-dialyzable	K₂O Exchange-able
				Milli-equivalents	*Milli-equivalents*	*Milli-equivalents*
4	K	Yes	6.72	39.7	0.125	0.109
		No	5.28	35.2	.111	.100
6	Check	Yes	6.92	36.1	.111	.090
		No	5.43	35.9	.075	.080
7	PK	Yes	6.87	36.7	.131	.092
		No	5.51	35.5	.102	.087
8	NK	Yes	6.71	36.0	.142	.098
		No	5.68	34.4	.126	.098
9	NPK	Yes	6.85	35.9	.146	.093
		No	5.56	34.7	.127	.070
10	2(NPK)	Yes	6.63	35.5	.155	.092
		No	5.59	34.5	.135	.081
11	Check	Yes	6.93	36.6	.110	.098
		No	5.42	32.9	.096	.063
14	P and manure	Yes	6.80	35.3	.132	.084
		No	5.47	33.8	.109	.082
15	2 (manure)	Yes	6.64	35.3	.165	.124
		No	5.53	35.2	.129	.115
16	Check	Yes	6.70	36.3	.122	.083
		No	5.44	36.1	.106	.083

DISCUSSION

While there is evidence that applications of potash fertilizers have increased the amount of exchangeable and electrodialyzable potassium, the most consistent effects have been brought about by liming.

If it be assumed that exchangeable or electrodialyzable potassium is a reliable index to availability, the logical conclusion to be drawn from the results is that liming has increased the availability of this element. This conclusion is obviously unjustified in view of the fact that, without exception, the total potash of the limed soils exceeds that of the unlimed, and the yields from the limed soils were generally greater. It has also been shown (6) that a soil with a comparatively low value for replaceable potassium may still supply sufficient potassium for high yields over a considerable period of time, whereas another soil of similar replaceable potassium value may be very deficient in potassium-supplying power.

Several investigators (2, 10, 11) have found that liming decreases the amount of potassium taken up by the crop, yet Lipman, Blair, and Prince (10) found that the total potash of the limed soils was, in general, lower than that of the corresponding unlimed soils. The differences could not be accounted for by the increased yields on the limed soils, but were attributed to soil variations and the release of potash by lime. However, Abel and Magistad (1) found that more potassium was removed by crops from the limed soil than from the unlimed. Analysis of hay from a few plots indicated that more potash was removed from the limed plots. Unfortunately, a sufficient number of analyses was not made nor were the yield data sufficiently differentiated to permit a calculation of the total potassium removed by the crops during the period involved. However, the results indicate the possibility that the losses by leaching may have been sufficiently lower in the limed soils to account for the higher total potassium content. This is supported by the results obtained by Abel and Magistad (1), who found that with a soil deficient in available potash the losses by leaching were greatly reduced while the removal by crops was increased by liming. The soil employed in the present investigation is low in exchange capacity and potash is the first limiting factor in crop growth.

In view of the results of recent investigations and the evidence obtained under the conditions outlined, it appears that there is a retention of potassium brought about by liming and that at least a part of the potassium is held in a form which, while available to plants, is incapable of entering the soil solution. This potassium is undoubtedly more available to plants capable of utilizing larger amounts of calcium, for as the calcium is removed by the plant the equilibrium is shifted in the direction in which potassium ions enter the system. This action takes place at the root-soil interface, and since the potassium is immediately utilized by the plant, the free soil solution is not enriched in potassium ions. Consequently, the losses of potassium by leaching are less than in the unlimed soils. The greater exchangeable and electrodialyzable potassium content of the limed soils may be accounted for in part by an increase in exchange capacity brought about by larger crop residues and higher pH.

SUMMARY

Fertilizer plot soils were subjected to electrodialysis and leaching with neutral ammonium acetate to determine the effect of fertilizers, cropping, and liming upon the amount of potassium removed by these methods.

Without exception, more potash was removed by electrodialysis from the limed soils than from the unlimed. The results for exchangeable potash were similar but of lesser magnitude.

Applications of potash were reflected to a greater degree by electrodialysis than by the exchange method.

Exchangeable and dialyzable potash were at a maximum in the plot receiving manure plus lime.

pH values were remarkably uniform throughout each series (limed and unlimed) of soils.

Without exception, total potash content of the soil was greater in the limed series.

For the soil investigated it appears that liming has conserved potash by reducing losses from leaching.

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AN EXPERIMENTAL STUDY OF THE RELATION BETWEEN THE UROPYGIAL GLAND AND VITAMIN D DEFICIENCY IN CHICKS [1]

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INTRODUCTION

The part played by the uropygial gland in the physiology of birds has been the subject of speculation for many years. Recent discoveries relating to vitamin D and the role of ultraviolet radiation in its elaboration have stimulated interest in the gland.

In 1913 Paris (12)[3] published the results of a very thorough experimental investigation of the structure and function of the uropygial gland. He, and others to whom he refers, concluded that the gland serves no indispensable purpose, for upon occlusion or extirpation of the gland no differences were observed between the experimental birds and the normal controls.

Rowan (13) noted that the preen gland is rich in cholesterol, and suggested that feather or feather oil may be a source of vitamin D.

Hou (4, 5, 6) investigated the relation of the uropygial gland to the production of vitamin D. His experimental evidence tended to show that the uropygial gland is the sole source of vitamin D precursor available to the bird. The results of further investigation (7) apparently substantiated this view. In a later publication, however, (8) he admitted that some other substance might be involved in vitamin D elaboration.

The fact that many birds lack the gland is not concordant with the idea that it performs a definite and necessary function.

The present investigation was undertaken with the relation between the uropygial gland and vitamin D production particularly in mind.

EXPERIMENTAL PROCEDURE

In the winter of 1930–31 and again in the fall of 1931, experiments were made to determine whether or not the removal of the uropygial gland from chickens would prevent the utilization of ultraviolet radiation in the production of vitamin D. The results of these two experiments indicated that the gland is dispensable in the calcium metabolism of the bird. The irradiated birds without the glands grew as well as the normal irradiated birds, and the ash content of their bones was as high.

On December 6, 1933, a third experiment was begun, which was carried out under carefully controlled conditions. One hundred Rhode Island Red chicks of medium weight were selected, and from 50 of these, the uropygial glands were extirpated. The combs were

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removed from all· of the birds to preclude the possibility of comb development interfering with the results (Maughan and Dye (*11*)).

Until the birds were put on experiment at 3 weeks of age, all of them were fed a mash ration containing 2 percent of cod-liver oil. This preliminary treatment is in accordance with the recommendation of Dove (*2, pp. 219–223*). The glands were removed when the birds were 2 weeks of age, and 1 week was allowed for recovery from the operation before the experimental treatment was begun. The birds were fed Hart's rachitogenic ration (*3*). This, the basic ration, was made up according to the following formula:

    59 parts ground yellow corn.
    25 parts standard wheat middlings.
    12 parts crude casein.
    1 part common salt.
    1 part precipitated calcium carbonate.
    1 part precipitated calcium phosphate.
    1 part dried yeast.

Some of the birds were subjected to ultraviolet prophylactic treatment and others to ultraviolet curative treatment. The prophylactic groups received 5 minutes daily ultraviolet irradiation instead of a fully protective dose of 20 minutes. If the presence of the uropygial gland were essential for normal calcification, the relation would be apparent at this lower level of protection.

Some control groups of birds received the rachitogenic ration supplemented with cod-liver oil. Other control groups were fed the basic ration without any vitamin supplement. Groups of chickens with the glands intact and groups with the glands removed were kept under similar conditions and subjected to similar treatments.

EXPERIMENTAL RESULTS

The results of the experimental treatments are shown in table 1. Each of the 10 groups consisted of 10 birds, some male and some female. Each bird was weighed at weekly intervals. The weekly weights are presented in figure 1, *A*, for the groups receiving prophylactic treatment, and in figure 1, *B*, for the groups receiving curative treatment.

TABLE 1.—*Treatment of chickens in the various groups of 10 birds each, and percentage ash of fat-free bones*

Group no.	Treatment [1]	Average percentage of ash based on fat-free bone
295	Gland intact; curative treatment; basic ration plus ½ percent Mazoil for 5 weeks, then supplemented with 20 minutes daily ultraviolet radiation for remaining 4 weeks of experiment	41. 61
300	Gland removed; curative treatment; basic ration plus ½ percent Mazoil for 5 weeks, then supplemented with 20 minutes daily ultraviolet radiation for remaining 4 weeks of experiment	42. 35
296	Gland intact; curative treatment; basic ration plus ½ percent Mazoil for 5 weeks, then ½ percent cod-liver oil substituted for Mazoil.[2]	44. 70
301	Gland removed; curative treatment; basic ration plus ½ percent Mazoil for 5 weeks, then ½ percent cod-liver oil substituted for Mazoil.	43. 82
293	Gland intact; control; basic ration plus ½ percent Mazoil.	36. 37
298	Gland removed; control; basic ration plus ½ percent Mazoil.	38. 14
294	Gland intact; preventive treatment; basic ration plus ½ percent Mazoil plus 5 minutes daily ultraviolet radiation.	40. 73
299	Gland removed; preventive treatment; basic ration plus ½ percent Mazoil plus 5 minutes daily ultraviolet radiation.	40. 71
297	Gland intact; preventive treatment; basic ration plus ½ percent cod-liver oil.	49. 17
302	Gland removed; preventive treatment; basic ration plus ½ percent cod-liver oil.	47. 75

[1] The basic ration was Hart's rachitogenic ration; Mazoil is a commercial corn oil.
[2] 4 of the birds died in the fourth week of the experiment from accidental causes.

In the prophylactic test, the irradiated birds with the glands removed gained less than the irradiated birds with the glands intact. These differences in growth, however, were too small to justify an assumption that the presence or absence of the gland was directly responsible for them.   In the curative test, the birds from which
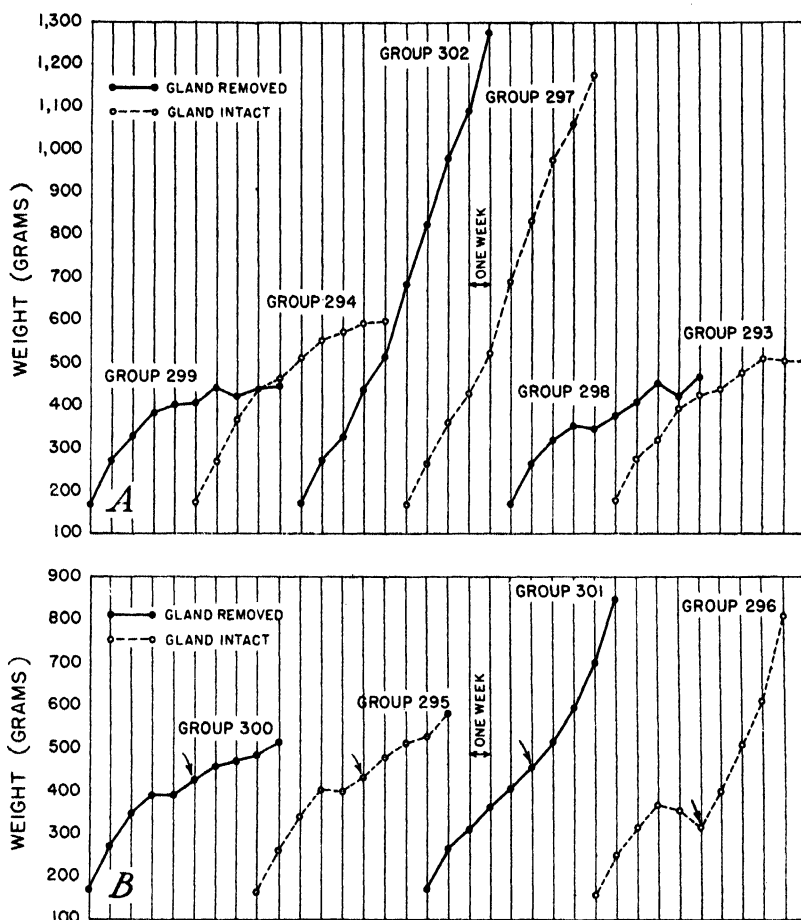
FIGURE 1.—Average weekly weight of chicks on preventive (*A*) and on curative (*B*) tests. Arrows in *B* indicate points at which daily irradiation with ultraviolet light was initiated for groups 295 and 300, and at which the cod-liver oil supplement was added to the basic (Hart's rachitogenic) ration consumed by groups 296 and 301.   Groups 293 and 298 (controls) were on the unsupplemented rachitogenic ration; this was supplemented, as explained in the text, with ultraviolet ray irradiations in groups 294, 295, 299, and 300, and with cod-liver oil in groups 296, 297, 301, and 302.   The uropygial gland was removed from all birds in groups 298 to 302 and was intact in groups 293 to 297.

the glands had been removed and which were subsequently allowed to become rachitic showed a definite improvement in growth upon the initiation of daily irradiation.

No differences in the calcification of the bones were apparent between comparable irradiated groups.   The ash content of the bones of the birds without the glands was as high as that of the bones of the birds with the glands.

DISCUSSION

It is difficult to reconcile the data here reported with the results of Hou's investigations. His experimental evidence tends to establish his premise that birds from which the uropygial glands are extirpated are unable to utilize ultraviolet radiation in the production of vitamin D. He concluded that the bird is dependent upon the uropygial secretion for vitamin D precursor.

The writer's experiments fail to substantiate this hypothesis. Since these experiments were completed, Knowles, Hart, Halpin, and Holmes, according to Clark (*1*), have completely refuted the conclusions of Hou. They were unable to discover any relation between the uropygial gland and the elaboration of vitamin D. In every case they found that the birds from which the glands had been removed grew as well as those in which the glands were intact. Knowles, Hart, and Halpin (*9*) published their data in detail, including bone analyses, which showed normal calcification in birds both with and without the gland. The writer's results, therefore, coincide with the conclusions of their investigations.

The origin of activatable ergosterol in the bird is still an open question. Maughan (*10*) implies that activation in chickens occurs in the circulating blood. Maughan and Dye (*11*) found that female chickens require double the irradiation required by males. They attributed this fact to the greater comb development of the males and the consequent larger area of uncovered body surface exposed to the rays. In the same paper Maughan and Dye report that exposure of the legs alone to ultraviolet radiation causes as rapid a recovery from rickets as total irradiation. Hou (*8*) stated his belief that the skin of the legs is too thick and the circulation too scanty to give credence to the hypothesis that the circulating blood contains the activatable ergosterol.

As far as the production of vitamin D is concerned the uropygial gland possibly augments the supply of ergosterol available in sufficient amounts from some other source. From the data presented the writer feels that it is safe to assume that the uropygial gland serves no indispensable function.

SUMMARY AND CONCLUSION

In a carefully controlled experiment the uropygial glands were extirpated from chicks in order to discover any relation that might exist between the presence of the gland and the ability of the birds to utilize ultraviolet radiation in the production of vitamin D.

Groups of birds with the glands removed and groups of birds with the glands intact were treated with ultraviolet radiation. Some groups were subjected to prophylactic and some to curative treatment. Control groups of birds, with and without glands, were fed the basic rachitogenic ration supplemented with Mazoil. Other control groups were given the basic ration supplemented with cod-liver oil.

The average weekly weights of the preventive groups, presented in graphic form, show small differences between the comparable groups receiving daily radiation.

It is evident from the graphic representation of average weekly weights that rachitic birds from which the uropygial glands had been removed definitely improved upon daily irradiation.

The bone analyses showed no differences in total ash content between groups with the glands removed and those with the glands intact.

It is concluded that, in chicks, the lack of the uropygial gland does not preclude the utilization of ultraviolet radiation in the production of vitamin D.

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OXIDATION-REDUCTION POTENTIALS OF SOIL SUS-PENSIONS IN RELATION TO ACIDITY AND NITRIFI-CATION[1]

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INTRODUCTION

Since Gillespie, (*2*),[2] in 1920, first measured the potential in a soil, using an indifferent electrode, numerous investigations have been made concerning the methods of measuring this oxidation-reduction potential. Several soil properties have been shown to be empirically associated with the potential, and numerous soil phenomena have been claimed, but not demonstrated to result from high or low potentials. For the most part, these investigations have been concerned with the methods of determination and the effects of artificial changes on the value of the potentials. There is a need for more fundamental study of the cause-effect relationships of the oxidation-reduction potential to other soil properties.

OBJECT OF EXPERIMENT

Of all the oxidation-reduction systems in the soil the most important, from an agronomic point of view, are those of carbon, nitrogen, and oxygen. The relation of the reversible oxygen system to the oxidation-reduction potential has already been established (*8*). That the carbon system is reversible has not been demonstrated, but Latimer and Hildebrand (*6*), in a table of potentials, indicate that the ammonia-nitrate system is reversible and has a definite potential at given concentrations. The statement of Heintze (*4*) that "in standardized media the form of the potential-time curve can serve to characterize groups of microorganisms or cellular processes, e. g., dentrification" leaves the conclusion to be drawn that the nitrogen system, when present in great enough concentration, should play a major part in establishing the potential of a soil.

A review of the literature has revealed only one study of soil oxidation-reduction potentials as they vary over a period of weeks; and that one (*7*) is an empirical study of potentials rather than an analysis of simultaneous changes which might cause the changes of potential. The objects of this experiment, therefore, have been (1) to study the changes in the oxidation-reduction potentials of suspensions of different soils over periods of time long enough to permit biological activity to cause significant changes in the nitrogen system, and thereby to ascertain whether the nitrogen system plays a part in establishing the potentials of soils; and (*2*) to note any changes in the oxidation-reduction potentials which may be ascribed to soil acidity or fertilizer treatment.

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PROCEDURE

Various soils were limed at abnormally high rates, and different form of nitrogen were applied. Periodic analyses of nitric and ammonic nitrogen, acidity, and oxidation-reduction potential were made over an 8-weeks' course of nitrification in the greenhouse.

The soils used were: (1) A well-drained sandy loam pasture soil; (2) the same soil in a natural waterlogged condition, showing the growth of sedges; (3) a gravelly sand, taken from a railroad fill; and (4) a black, sandy soil taken from a swamp.

About 400 pounds of each moist soil was collected, and after the soils had dried for 2 to 3 weeks, the clods were broken up and the samples were sifted twice through a three-mesh sieve, mixed several times, and divided into two parts. To one lime was added at the rate of $7\frac{1}{2}$ tons per acre (58.5 percent Ca $(OH)_2$, 41.5 percent $CaCO_3$); to the other no lime was added. One week later, both the limed and the unlimed portions were again subdivided into four 8-kg parts each. Nitrogen was then applied as explained in table 1, after which the 32 resulting samples were placed, to a depth of about 4 inches, in wooden boxes 1 foot square by 6 inches deep, so constructed as to leave a quarter-inch opening along the bottom, which was covered with a paper towel. The boxes were placed on a greenhouse bench and watered at least once a week; the moisture content was kept between 3 and 25 percent.

Samples were taken at the time of nitrogen application and at intervals of approximately 2 weeks, for the determination of moisture, nitrate nitrogen, ammonia nitrogen, acidity, and oxidation-reduction potential.

Determinations of pH were made with the quinhydrone electrode. About 15 g of air-dry soil, 0.05 g of quinhydrone, and a 1: 2 soil-water ratio were used.

TABLE 1.—*Description and treatment of soils*

Soil no.	Description	Lime applied	Nitrogen applied
100	Well drained, fine sandy loam, from Brooks' farm, Massachusetts State College.	None	None.
101		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
102		do	1 g dried blood per 100 g soil (10 percent N).
103		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
110		$7\frac{1}{2}$ tons per acre	None.
111		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
112		do	1 g dried blood per 100 g soil (10 percent N).
113		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
200	Poorly drained, fine sandy loam, from Brooks' farm, Massachusetts State College.	None	None.
201		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
202		do	1 g dried blood per 100 g soil (10 percent N).
203		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
210		$7\frac{1}{2}$ tons per acre	None.
211		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
212		do	1 g dried blood per 100 g soil (10 percent N).
213		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
300	Gravelly sand fill, from Tillson farm, Massachusetts State College.	None	None.
301		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
302		do	1 g dried blood per 100 g soil.
303		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
310		$7\frac{1}{2}$ tons per acre	None.
311		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
312		do	1 g dried blood per 100 g soil.
313		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
400	Black, sandy swamp soil, from Tillson farm, Massachusetts State College.	None	None.
401		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
402		do	1 g dried blood per 100 g soil.
403		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.
410		$7\frac{1}{2}$ tons per acre	None.
411		do	30 mg NH_3-N per 100 g soil, as $(NH_4)_2SO_4$.
412		do	1 g dried blood per 100 g soil.
413		do	30 mg NO_3-N per 100 g soil, as $NaNO_3$.

Nitrate nitrogen determinations were made colorimetrically by the phenoldisulphonic method.   Ammonia nitrogen determinations were made according to Harper's method (*3*).

The oxidation-reduction potentials were determined by a modification of Willis' method (*8*).   The modification consisted chiefly in the omission of the displacement of air with nitrogen, which Herzner (*5*), Brown (*1*), and Heintze (*4*) have shown to be unnecessary.   An experiment conducted by the senior writer in which the two methods were compared showed that the results by the modified method can be rather well duplicated on different samples of the same soil, and that the displacement of air is not necessary.

Fifty grams of moist soil were placed in a 250-cc flask with an equal weight of distilled water.   The flask was stoppered and shaken at room temperature (about 28° C.) for 2½ to 3 days.   The contents of the flask were then transferred to a 250-cc three-necked Woulff bottle, two bright platinum electrodes were inserted and the bottle was placed in a constant-temperature water bath at 35° C.   Connection to a saturated calomel half-cell was made with a 3-percent agar-saturated potassium chloride bridge.   Within 15 minutes of contact of the electrodes with the soil suspension, the potential of the resultant cell,

Hg | HgCl (sat.), KCl | Agar–KCl | soil suspension | Pt,

was measured electrometrically on a Leeds & Northrop portable acidity meter.   The duplicate electrodes always checked within 5 millivolts and generally within a narrower range.

Between determinations on different soils, the agar bridge was rinsed with saturated KCl; between weekly runs, it was set in saturated KCl to prevent desiccation.   Before and after each determination on different soils, the platinum electrodes were rinsed with water, then with alcoholic alkali, alcohol, and water.   Between weekly runs, the electrodes were set in distilled water.

RESULTS

The results of the experiment are given in tables 2 and 3.   The results from six representative soils are shown graphically in figure 1. Nitric and ammonic nitrogen were expressed as parts of nitrogen per 10,000 of moisture-free soil.   Acidity was expressed in pH values. Since the values of soil potentials found by the majority of previous investigators seem to indicate that they have been expressed as the potentials of the half-cell in tenths of volts,

Soil suspension | Pt,

the potentials observed in this experiment have been expressed in the same manner, for purposes of comparison.   Although this is not in accord with the convention followed in most textbooks, viz, the expression of such potentials as those of the cell above, reversed, there is no essential difference as long as it is specified which half-cell is being spoken of, for in either case the interpretation is that the oxidation-reduction system of the soil is more oxidative than the standard hydrogen electrode.

TABLE 2.—*Concentrations of ammonic nitrogen and of nitric nitrogen in soils* [1] *on different dates*

Soil no.	Feb. 7		Feb. 23		Mar. 10		Mar. 20		Apr. 4	
	NO$_3$-N	NH$_3$-N	NO$_3$-N	NH$_3$-N	NO$_3$-N	NH$_3$-N	NO$_3$-N	NH$_3$-N	NO$_3$-N	NH$_3$-N
	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
100	15	38	23	30	52	24	67	14	62	4
101	15	344	16	340	34	292	26	308	103	90
102	66	72	62	431	106	432	111	418	182	556
103	300	37	163	38	444	50	361	52	487	46
110	17	36	47	30	71	17	44	29	89	7
111	16	283	220	47	353	23	313	72	370	37
112	13	61	177	377	236	307	390	195	589	207
113	275	31	483	44	487	38	265	5	470	7
200	48	31	79	49	80	51	85	43	82	33
201	32	326	32	310	69	406	21	331	88	369
202	43	60	82	----------	208	288	246	417	285	484
203	278	31	353	56	462	67	306	63	461	74
210	46	31	51	47	164	23	113	6	175	3
211	26	278	193	79	523	47	388	9	382	3
212	47	58	186	351	551	121	471	22	881	43
213	321	30	326	70	259	40	382	31	500	28
Soil no.	Feb. 15		Feb. 28		Mar. 16		Mar. 28		Apr. 10	
300	10	14	14	75	23	7	36	8	49	6
301	10	334	15	340	23	236	41	194	63	247
302	10	55	27	425	68	432	122	357	172	444
303	360	18	228	66	324	32	315	27	324	37
310	10	21	27	72	116	20	135	23	201	5
311	9	321	20	235	192	135	263	78	520	40
312	10	70	23	463	61	349	52	325	240	363
313	312	21	282	105	340	63	471	56	545	41
400	6	34	5	82	14	64	22	64	73	36
401	3	258	Trace	283	Trace	360	Trace	379	Trace	477
402	3	82	12	595	15	686	11	689	53	783
403	313	31	294	79	316	87	396	96	358	114
410	3	46	20	85	97	31	129	10	108	28
411	3	333	19	357	148	161	271	111	141	76
412	4	98	42	552	595	157	321	178	807	9
413	307	41	220	87	385	58	554	21	328	10

[1] See table 1 for soil treatments; all determinations calculated on the basis of moisture-free soil.

TABLE 3.—*Acidity and oxidation-reduction potentials* [1] *of soil* [2] *suspensions on different dates*

Soil no.	Feb. 11		Feb. 24		Mar. 11		Mar. 20		Apr. 4	
	pH	E$_h$	pH	E$_h$	pH	E$_h$	pH	E$_h$	pH	E$_h$
		Volt		Volt		Volt		Volt		Volt
100	5.47	+0.488	5.38	+0.532	5.21	+0.529	4.90	+0.571	5.19	+0.600
101	5.46	+.493	5.31	+.532	5.21	+.530	4.83	+.603	4.89	+.615
102	5.88	+.486	5.79	+.488	5.64	+.500	5.44	+.452	5.40	+.499
103	5.43	+.484	5.38	+.571	5.17	+.544	5.01	+.583	5.14	+.585
110	7.31	+.386	7.26	+.435	6.47	+.448	6.56	+.434	5.98	+.486
111	7.74	+.386	7.10	+.431	6.73	+.447	6.97	+.441	6.09	+.485
112	7.84	+.040	7.70	+.343	7.38	+.365	7.11	+.419	6.61	+.467
113	7.62	+.387	7.65	+.433	7.46	+.441	7.44	+.442	7.38	+.487
200	5.48	+.479	5.36	+.560	5.36	+.558	5.07	+.576	5.25	+.575
201	5.42	+.479	5.35	+.561	5.34	+.556	4.98	+.589	5.05	+.589
202	5.87	+.462	5.95	+.494	5 29	+.556	5.02	+.569	5.19	+.583
203	5.43	+.477	5.33	+.560	5.22	+.563	5.01	+.582	5.14	+.583
210	7.30	+.357	7.55	+.448	6.93	+.463	7.24	+.466	7.24	+.473
211	7.48	+.362	7.31	+.454	6.90	+.474	7.09	+.489	7.31	+.501
212	7.91	+.085	7.50	+.358	7.05	+.450	7.03	+.490	6.98	+.507
213	7.64	+.356	7.75	+.442	7.53	+.449	7.62	+.481	7.65	+.487
Soil no.	Feb. 15		Mar. 2		Mar. 16		Mar. 28		Apr. 10	
300	5.28	+0.523	5.04	+0.558	4.65	+0.553	4.47	+0.581	4.85	+0.614
301	5.11	+.524	4.89	+.575	4.51	+.575	4.27	+.597	4.60	+.617
302	5.32	+.471	5.47	+.093	5.80	+.343	4.98	+.457	5.00	+.403
303	5.06	+.560	4.87	+.575	4.63	+.568	4.49	+.568	4.84	+.533
310	8.07	+.328	7.46	+.389	6.87	+.392	6.70	+.435	7.00	+.513
311	8.16	+.321	7.74	+.388	7.23	+.374	6.90	+.426	6.30	+.503
312	8.17	+.020	8.07	+.030	7.42	+.030	7.35	+.038	7.52	+.377
313	8.09	+.318	7.80	+.400	7.55	+.382	7.03	+.415	7.54	+.458
400	5.15	+.491	5.30	+.537	4.82	+.540	4.74	+.556	5.02	+.590
401	5.16	+.499	4.98	+.544	4.71	+.564	4.68	+.535	5.00	+.570
402	5.39	+.391	5.52	+.265	5.50	+.306	5.42	+.368	5.49	+.477
403	5.16	+.504	4.95	+.535	4.81	+.536	4.70	+.543	5.00	+.560
410	7.01	+.378	6.47	+.429	6.00	+.447	6.18	+.476	6.08	+.496
411	7.64	+.372	6.91	+.440	6.55	+.458	6.15	+.460	6.21	+.500
412	7.68	+.103	7.33	+.191	6.53	+.355	6.32	+.403	6.10	+.517
413	7.64	+.387	7.05	+.413	7.06	+.419	6.67	+.479	6.71	+.499

[1] Expressed as the potential of the half-cell, | soil suspension | Pt, at 35° C.
[2] See table 1 for soil treatments.

Nitrification, changing acidity, and varied nitrogen treatments were considered as possible causes of changes of potential. For comparing nitrification and potential, two methods were used: (1) The increase of nitrate nitrogen over the original determination, the decrease of ammonia nitrogen, and the sum of these two values, considered as
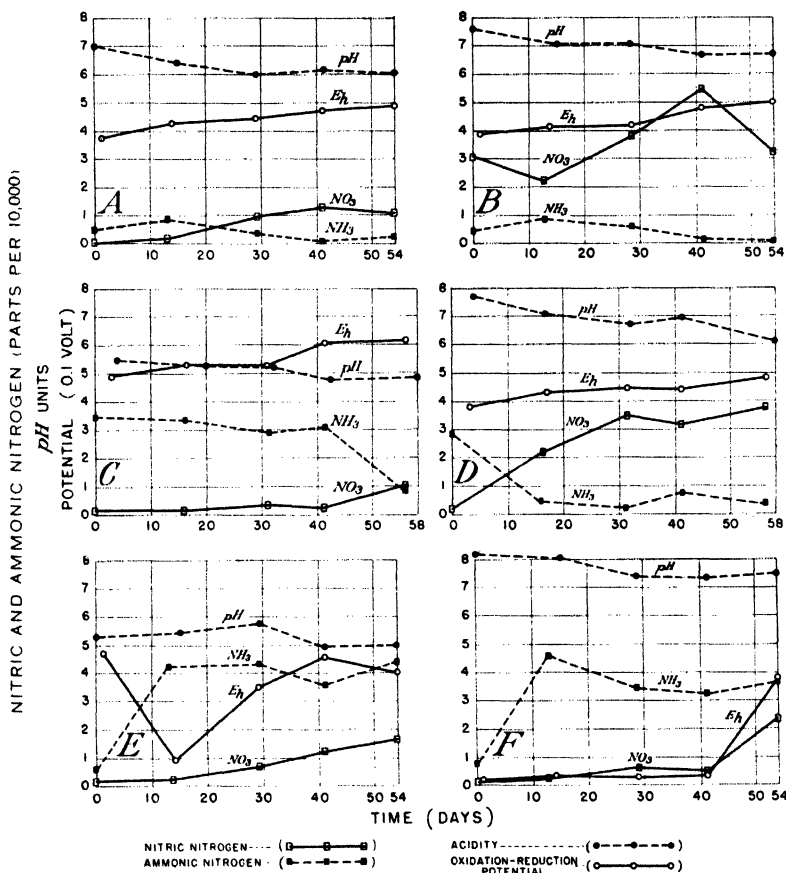
FIGURE 1.—Nitric and ammonic nitrogen in parts per 10,000 of moisture-free soil, acidity as pH, and half-cell potential, of six soils during the experimental period: *A*, Soil 410, poorly drained, treated with lime, no nitrogen, in tenths of volts; *B*, soil 413, poorly drained, treated with lime and NaNO₃; *C*, soil 101, well-drained, treated with (NH₄)₂SO₄, no lime; *D*, soil 111, well-drained, treated with lime and (NH₄)₂SO₄; *E*, soil 302, well-drained, treated with dried blood, no lime; *F*, soil 312, well-drained, treated with lime and dried blood.

giving a measure of the amount of nitrification, were compared with the simultaneous increase of potential over its initial value; (2) values of log $(NO_3-N)/(NH_3-N)$ were calculated at each determination, and plotted on scatter diagrams against simultaneous values of potential. In comparing potentials with acidity, the coefficients of correlation and regression were calculated as explained below. In the comparison of potential with nitrogen treatment, the initial potentials of nitrogen-treated soils were compared with the potentials of their corresponding no-nitrogen treatment.

CHANGES OF POTENTIAL WITH RELATION TO NITRIFICATION

In general, since nitrification took place at the expense of the ammonia content of the soils, the NO_3–N and NH_3–N concentrations showed a rough inverse trend, except in those soils where there was considerable ammonification of added or already present organic matter.

The increases of nitrate content over the initial determination for each soil were calculated at 2, 4, 6, and 8 weeks. In like manner, the decreases of ammonia content and the values of (increase nitrate + decrease ammonia) were calculated. These measures of nitrification were compared with the increases of potentials observed at corresponding times.

This method of comparison showed no definite relationship within the individual soils. In nearly all soils the potentials showed steady increases. But as the potential of a soil increased, the nitrification often decreased. For example, in soil 203 the increases in potential over the initial value were 83, 86, 105, and 106 mv, while the corresponding nitrate increases were 75, 184, 28, and 183 p. p. m., the ammonia decreases were -25, -36, -32, and -43 p. p. m., and the values of (increase NO_3 + decrease NH_3) were $+50$, $+148$, -4, and $+140$ p. p. m.

When the increase for any limed soil was compared with the simultaneous increase for its corresponding unlimed soil, however, there appeared to be a more definite relationship. In all but 9 of the 64 simultaneous comparisons of increase of potential, the increase was more positive in the limed soil than in the unlimed. Likewise, in all but 10 and 12 comparisons, respectively, the increase of nitrate and the decrease of ammonia were more positive in the limed soil than in the corresponding unlimed soil. This relationship was merely qualitative, as the magnitudes of increases of potential were not proportional to increases of nitrate.

The final criterion, however, of the effect of any oxidation process on potentials must be the variance of potential with changing ratios of oxidant to reductant. Bearing this in mind, and the equation for the potential of any reversible oxidation-reduction system

$$E_h = E_o + \frac{RT}{nF} \log \frac{\text{(oxidant)}}{\text{(reductant)}},$$

values of $\log \left(\frac{NO_3-N}{NH_3-N} \right)$ were calculated for each simultaneous determination. With these values as abscissae and the corresponding observed values of E_h as ordinates, scatter diagrams were constructed for various combinations of soils, as follows: (1) All soils; (2) all unlimed soils; (3) all limed soils; (4) all limed soils except those receiving dried-blood treatment; (5) all different lime and nitrogen treatments of each of the four original soils; (6) each similar nitrogen treatment of all soils.

The only combination in which the points appeared to be grouped along a straight line was combination 4, of all limed soils except the dried-blood-treated. In this combination there appeared to be a slight positive correlation of E_h to $\log (NO_3-N)/(NH_3-N)$. The points for the dried blood treatments, however, after the first one or two determinations, also fell fairly well within the same range. This strengthened the proposition that oxygen depletion was effec-

tive in lowering the potentials during the initial stages, when decompo-sition of organic matter and ammonification were rapid.

To summarize: Changes in potential were more closely correlated with changes in acidity than with changes in the nitrogenous constit-uents. The addition of large amounts of nitrate nitrogen or am-monia nitrogen had very little effect on the potentials of suspensions of the soils. The apparent correlation of potentials with log (NO_3/NH_3) held only in limed soils, where nitrification was very active and where there were large changes in acidity.

As a general rule, the process of ammonification is considered as an alkalizing process, and that of nitrification as an acidifying process. The results of this experiment were interpreted as being in accord with this relationship, viz, when the microbiological processes of ammonification and nitrification were active in the soils, a change was reflected in the potentials observed on suspensions of the soils. The changes in potential were more closely correlated with changes in acidity than with changes in the nitrogenous constituents, suggest-ing that the resultant acidity or alkalinity, rather than the presence of nitrogen as ammonia or nitrate, was the cause of changes in potential.

CHANGES OF POTENTIAL WITH RELATION TO CHANGING ACIDITY

The observations of Herzner (5), Willis (8), and Heintze (4), that the potential is a direct function of acidity were remarkably well verified by the results of this experiment. This relationship held equally well for changes of acidity produced by liming and for those produced in the course of time along with changes in the nitrogenous constituents.

Eliminating the dried blood treatments, in which the pH–E_h re-lationship seemed to be complicated by the rapid decomposition of organic matter, the remainder of the simultaneous pH and E_h readings showed a decidedly negative correlation. The correlation coefficient of these variables at 0, 2, 4, 6, and 8 weeks was, respectively, -0.97 ± 0.0081, -0.96 ± 0.0108, -0.92 ± 0.0211, -0.74 ± 0.0623, and -0.90 ± 0.0262. When combined without respect to time, all the pH and E_h readings except those of the dried-blood treatments showed a gross correlation coefficient of -0.85 ± 0.0171. The regression coefficient, which is a measure of the increase of po-tential with a unit decrease of pH, was, at the same intervals, 0.0594, 0.0575, 0.0553, 0.0381, and 0.0479. The regression coefficient of all the readings except those of the dried blood treatments, combined without respect to time, was 0.0540. These figures compare rather favorably with the figure 0.0611, which is the theoretical regression coefficient of the potential of a monoelectronic oxidation-reduction system with pH, at 35° C. Although this may not be conclusive, since the homogeneity and size of the population have not been statis-tically validated, it seems to indicate that the hydrogen-hydrogen ion system is very important in determining the magnitude of the oxidation-reduction potentials of soils.

CHANGES OF POTENTIAL DUE TO APPLICATION OF NITROGEN CARRIERS

The potentials of suspensions of different nitrogen treatments of the same soil, when compared with that of the no-nitrogen treatment, showed only slight, inconsistent changes in the cases of the $NaNO_3$ and the $(NH_4)_2SO_4$ treatments. This lack of change confirmed the idea stated above that changes in ammonia and nitrate content effect

changes of potential only as these changes alter the acidity of the soil. The treatments with dried blood, on the other hand, resulted in, marked decreases of potential, particularly at high pH values. In these soils there was a rapid decomposition of the added organic matter, as was indicated by the large rises in ammonia content during the first 2 weeks of the experiment. During this period, the potentials of suspensions of the dried-blood-treated soils were from 50 to 350 mv lower than would be expected from the pH–E_h relationship which has been demonstrated for the other soils. It has been shown by Willis [3] that oxygen depletion should cause a drop in potential if the pH remains the same. It is suggested that in such cases as these dried-blood-treated soils, the rapid decomposition of organic matter was accompanied by oxygen depletion, which caused the drops in potential.

SUMMARY

By means of a greenhouse study of four soils from different drainage conditions, at different lime levels, and with different nitrogen fertilization, the relationships of the oxidation-reduction potentials of suspensions of these soils to nitrification, acidity, and kind of nitrogen carrier were investigated.

It was found that any change of potential with nitrification is secondary to the effect of nitrification on pH.

The inverse relationship found independently by Herzner, Willis, and Heintze to exist between pH and E_h was verified.

The addition to the soils of $(NH_4)_2SO_4$ or $NaNO_3$ was found to have no constant effect on the potentials of the suspensions.

The rapid decomposition of organic matter brought about a marked fall of potential. This fall was apparently caused by oxygen depletion.

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THE NATURE OF RESISTANCE OF FLAX TO MELAMPSORA LINI [1]

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INTRODUCTION

It has been recognized for some time that the culture of flax, *Linum
usitatissimum* L., may in certain years be seriously impaired by flax
rust (*Melampsora lini* (Pers.) Lév.). *Melampsora lini* has been re-
ported under one name or another from North America, South
America (Argentina), Europe (Great Britain, France, Germany,
Russia, Italy, Bulgaria, Austria, Switzerland, and Spain), Asia
(India, Asia Minor, and Siberia), Australia, China (Manchuria and
Mongolia), and Japan (Sakhalin, Hokkaido, and Northern Honshu)
(*20*).[3] Flax rust may therefore be considered to occur wherever flax
is grown.

In the United States flax rust has been reported to be epidemic in
the seed-flax regions of Minnesota, North Dakota, South Dakota,
Iowa, Montana, and Wisconsin,[4] while it has been reported as en-
demic in the fiber-flax regions of eastern Michigan and the Willa-
mette Valley of Oregon (*35*). In Canada the disease has been re-
ported from the seed-flax centers of Alberta, Manitoba, Saskatche-
wan, Ontario, and Quebec.

Flax rust generally causes a partial or complete defoliation of
seed-flax stands and in most cases ultimately causes a considerable
reduction in yield. Most of the North American varieties of seed
flax are not, as a rule, killed by rust under field conditions, although
a few of the imported selections may be completely destroyed. In
general, the average annual loss of seed flax from flax rust in North
America varies from 1 to 10 percent of the total seed crop, although
in 1928 losses as high as 35 percent were reported from several flax
sections of North Dakota.

While various investigators have worked on the general aspects
of flax rust, its epidemiology and control, little or no work appears
to have been done on the nature of varietal resistance. The object

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[2] The writer expresses his appreciation to Dr. E. C. Stakman for helpful advice, sug-
gestions, and criticisms, and for the continual interest which served to stimulate and
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stomatal study and histological work; to C. C. Allison for the use of flax varieties at
Coon Creek and for assistance in field experiments; and to Dr. J. J. Christensen for
numerous kindly suggestions and criticisms.
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U. S. Dept. Agr. Bur. Plant Indus., Plant Disease Reptr. Sup. 35; 294–295, 1924; 40;
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[Mimeographed.]

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of this investigation was to determine, as far as possible, the exact nature of varietal resistance in cultivated flax to flax rust, with the aim of providing data which might be of assistance to plant breeders in the production of rust-resistant varieties.

During the past few years the nature of resistance of plants to certain fungus pathogens has been studied extensively, and three main theories of resistance have been developed: (1) The morphological theory, which affirms that certain morphological or anatomical features of the host plant either preclude the entrance of the pathogen into the plant or limit its development once it has gained entrance; (2) the functional theory, which suggests that stomatal movements of the host plant may play an important part in precluding or restricting the entrance of certain pathogens; and (3) the physiological theory, which postulates that certain inherent physiological properties of the host plant operate in determining the degree of resistance or susceptibility of the host to the fungus pathogen.

The present discussion approaches the problem from the angles suggested by the foregoing theories and is therefore divided into a morphological study, a stomatal study, and a physiological study.

REVIEW OF PREVIOUS INVESTIGATIONS

Arthur (*3, p. 201*), in 1907, demonstrated that *Melampsora lini* was euautoecious but did not find it specialized for particular species of *Linum*. Fromme (*14*), in 1912, described and figured the cytology of the causal organism, while Tobler (*42*), in 1920, studied the histological effects of the pathogen upon the host tissues. In Ireland the general aspects of flax rust have been studied by Pethybridge et al. (*26, 38, 39*). In 1926 Hart (*16*) studied the factors conditioning the development of flax rust. Henry (*19*), in 1928, studied the various phases of flax-rust control and demonstrated the possibility of control by the production of rust-resistant varieties through hybridization.

Bolley (*4, 5*), as early as 1903, noticed that several strains of cultivated flax reacted differently to flax rust, and obtained several rust-resistant strains by means of mass selection. Sydow and Butler (*41*) reported that European varieties of flax remained free from rust in India, while indigenous varieties were severely attacked. Eriksson (*12*), in 1912, noted that different varieties of flax had varying degrees of susceptibility. Butler (*6*) mentioned a variety of flax from Pusa, India, which had never been attacked successfully by flax rust. Westerdijk (*48*), in 1918, stated that flax rust was known in the Netherlands only on the white-blossom varieties.

Girola (*15*) pointed out that "Lino mal abrigo" (a group of Argentine varieties of flax having medium-sized seeds) was noted for its resistance to rust. Howard (*21, pp. 382–383*) reported that of the three classes of flax varieties, large-, medium-, and small-seeded, grown at Pusa, India, varieties of the last class did not suffer from rust. Dorst (*11*), in 1923, isolated rust-resistant strains of fiber flax in the Netherlands, and Dillman (*10*) has reported that certain varieties of Argentine flax are immune from rust when grown in the United States. Henry (*19*), working at the Minnesota station, conducted varietal tests for several years and noted distinct differences

in the resistance of different varieties. He isolated immune pure lines from commercial, large-seeded Argentine varieties, and also reported that the variety Ottawa 770B C. I. 355 was consistently immune in all of his tests. In addition, Henry found immune strains in the variety Williston Golden C. I. 25 and in several selections obtained from India.

COURSE OF DEVELOPMENT OF THE PATHOGEN IN SUSCEPTIBLE AND RESISTANT VARIETIES

DEVELOPMENT OF FLAX RUST WITHIN THE TISSUES OF A SUSCEPTIBLE HOST

While the sexual fusions and cytological phenomena of *Melampsora lini* have been investigated by Fromme (*14*), Moss (*36, 37*), and Allen (*2*) a survey of the pertinent literature has failed to reveal a complete and detailed description of the histological development of the casual organism within the tissues of a susceptible host. Partly for this reason it was considered desirable to study histologically and depict diagrammatically the development of flax rust within the tissues of a susceptible host, namely, Winona flax. The development of the pycnial and aecial stages has been studied and illustrated in considerable detail by Fromme (*14*); hence, the writer studied the development of the pathogen from the time of penetration of the urediospore germ tube into the host until the formation of the morphologically mature telium.

Plants of Winona flax raised in pots in the greenhouse were inoculated by applying urediospores to the surfaces of the leaves and stems by means of a scalpel, and incubated for 48 hours. Leaf and stem material was removed from these inoculated plants at definite intervals and killed in medium chromo-acetic killing and fixing solution. The material was passed through the butyl alcohol series (*49*), imbedded, sectioned, and stained. The triple stain combination of safranine, gentian violet, and orange G employed by Wellensiek (*47*) was used.

INFECTION PHENOMENA

It has been shown by Hart (*16*) that the germ tubes of *Melampsora lini* grow toward the stomata of the host plant, form appressoria, enter through the stomatal pores, and subsequently develop substomatal vesicles which proceed to the formation of a mycelium. In this investigation attempts were made to obtain sections of stem material showing penetration of the urediospore germ tubes through stomata, but without success. However, definite cases of penetration into leaf material were readily obtained. By stripping off portions of the epidermis of inoculated leaves and staining with cottonblue stain it was possible to study the infection phenomena in surface view (fig. 1, *A*). The germ tube of the urediospore grows upon the surface of the leaf, in some cases branching rather frequently, but eventually some of the germ tubes grow over an open stoma. The tip of the germ tube, or the apical portion of a branch of the tube, in most cases swells to form a globoid, bladderlike structure, the "appressorium", situated immediately over the stoma. The function of the appressorium appears to be to provide, by virtue of its increased area of attachment, greater purchase for the germ tube while

the latter sends its infection hypha between the guard cells of a stoma. In cross section, the germ tube is observed to follow the contours of the leaf epidermis very closely (fig. 1, *B*). An infection peg is sent out from the appressorium and pushes its way between the guard cells of the stoma, and swells to a small, globoid vesicle in the substomatal cavity, and then proceeds to the formation of an infection hypha.
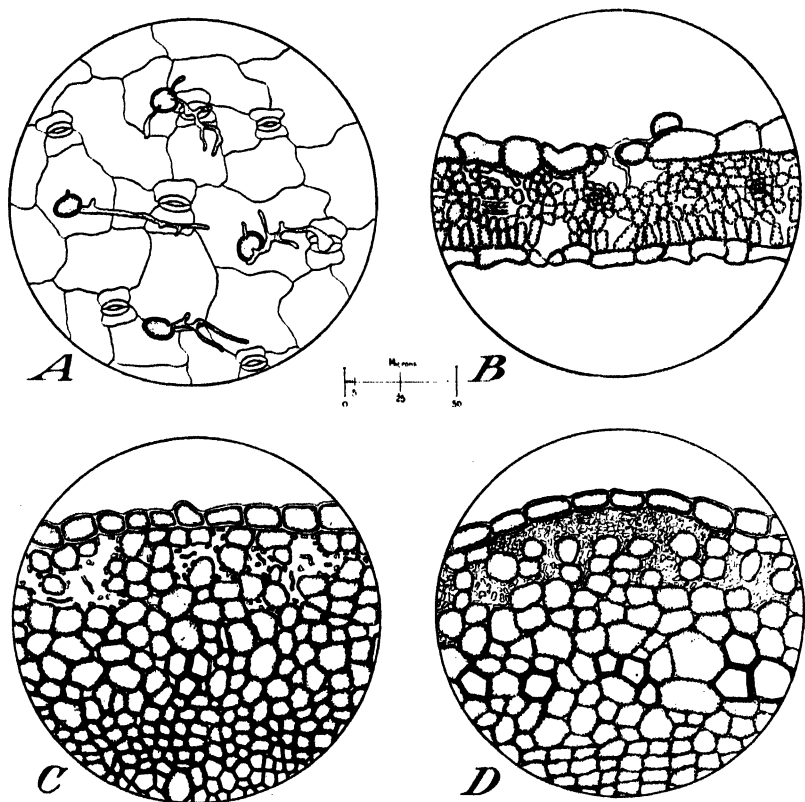
FIGURE 1.—Development of flax rust within the tissues of a susceptible host. (Camera lucida drawings) : *A*, Surface view of leaf epidermis, showing germination of uredio- spores, appressorium formation, and penetration of germ tubes through stomata ; *B*, transverse section of leaf, showing penetration of uredial germ tube through an open stoma and the formation of an infection hypha ; *C*, transverse section of a stem, showing development of rust hyphae in cortical tissues of host ; *D*, transverse section of stem 7 days after inoculation, showing formation of pustule stroma resulting in a bulging of the epidermis.

DEVELOPMENT OF THE UREDIUM

While definite cases of penetration were not obtained with stem material, very early stages of infection were observed. The first histological evidence obtained for infection in stem material was the occurrence of numerous isolated hyphae in the outer cortical tissues, appearing in transverse section as small spherical bodies (fig. 1, *C*). Approximately 7 days after inoculation a definite stroma is formed in the cortical tissues of the stem (fig. 1, *D*). The hyphae have greatly increased in number and have become aggregated

into a compact, stromalike body, which causes the epidermis to bulge noticeably. Approximately 10 days after inoculation a palisadelike layer of binucleate cells was observed immediately below the epidermis (fig. 2, *A*). The binucleate cells constitute the initial cells of the uredium and are supported by a stroma of small,
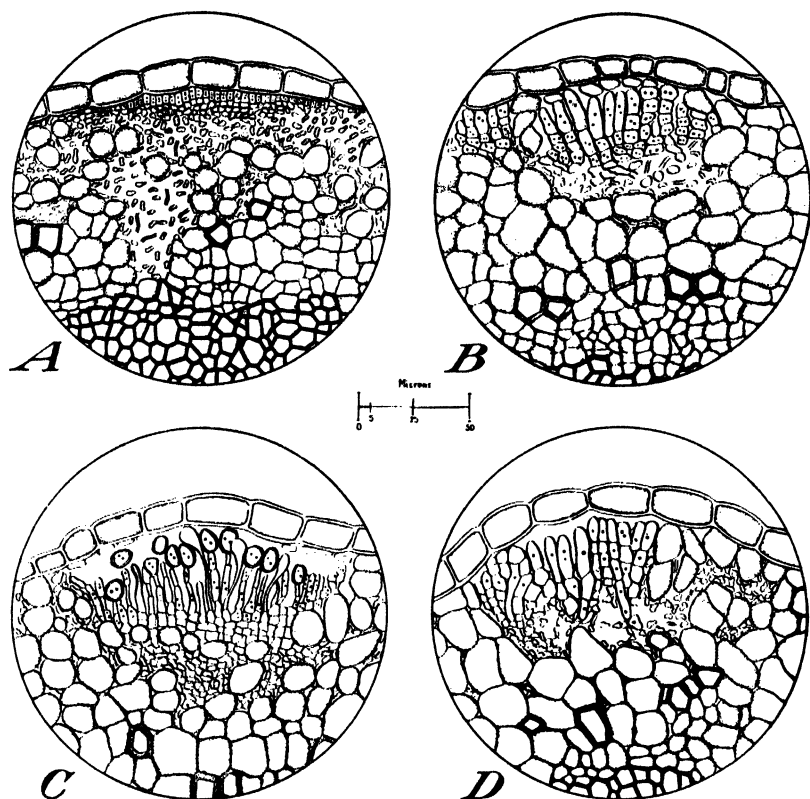
FIGURE 2.—Further development of flax rust within the tissues of a susceptible host. (Camera lucida drawings.) *A*, Transverse section of stem 10 days after inoculation, showing development of palisadelike layer of binucleate urediospore initial cells. *B*, transverse section of stem 12 days after inoculation, showing development of very young uredial sorus. The binucleate initial cells on the left side of the sorus have divided to give rise to the peridial, intercalary, and basal cells. On the extreme right of the sorus the basal cells have in turn divided to produce the apical spore initial cell, the still unelongated pedicel cell, and the basal mother cell. The evanescent peridial cells are also shown. *C*, transverse section of mature uredial sorus, showing fully formed urediospores with echinulate walls attached to the elongated pedicel cells. Intermingled with the pediceled urediospores the capitate paraphyses may be observed. *D*, transverse section of stem 21 days after inoculation, showing initial of telial sorus in the form of a palisadelike layer of cells. In center of sorus the initial cells have divided to form the apical teliospores and the basal cells.

rounded, fairly closely aggregated cells. In this stage the rust mycelium has become quite extensive and occurs throughout the cortical region of the stem, in some cases penetrating as far as the vascular ring, but in no case was it observed to affect or penetrate the vascular system. Approximately 12 days after inoculation the binucleate cells had developed into mature urediospores. Each cell of the palisade layer divided into three cells, the peridial, intercalary, and basal cell. The basal cell divides and thus gives rise

to spore initials, which, by their continued growth, result in the destruction of the intercalary cells. The peridial cells are usually evanescent but may persist under the still unruptured epidermis as scattered cells. The basal cell now divides twice, giving rise to the spore initial, the spore pedicel cell, and the mother basal cell. A uredium in this stage of development is illustrated in figure 2, *B*. Immediately beneath the epidermis five peridial cells are shown, the intercalary cells having disappeared. On the right of the uredium the three basal cells illustrated have completed their divisions, producing the apical spore initial, the still unelongated spore pedicel cell, and the mother basal cell. The six initials on the extreme left of the uredium are slightly less mature and the spore initials have not as yet commenced to round up. All the cells at this stage of development are binucleate. In the mature uredium the urediospores have developed their echinulate outer walls and are attached to the greatly elongated pedicels (fig. 2, *C*). Intermingled with the urediospores are the long, slender, capitate paraphyses, which tend to arch over the spores. In this stage the stroma is well developed and the epidermis greatly bulged. The spore pedicels continue to elongate and the spores to increase in size until sufficient pressure is exerted to rupture the epidermis and allow the spores to escape. In certain preparations it was noticed that many of the spores were flattened in contact with the epidermis, indicating that considerable pressure must have been applied before the epidermis was ruptured and the urediospores liberated.

DEVELOPMENT OF THE TELIUM

Approximately 3 weeks after inoculation the initial of the telium makes its appearance. The telium may be initiated in an old uredium or it may arise independently in the cortical (or mesophyll) region of the host stem (or leaf). The telium arises in a manner similar to the uredium, small primordia developing below the epidermis and dividing to form a palisade of vertically arranged hyphal cells (fig. 2, *D*, extreme right). The initial cells are binucleate and become divided by a transverse septum into the lower basal cell and the upper teliospore (fig. 2, *D*, center of sorus). The teliospores become laterally compacted into a waxy sorus, which may become very extensive, a single telial layer in some cases extending half-way round the stem (fig. 3). The individual teliospores are embedded at their tips in a deep-staining waxy material, and quite commonly a second layer of teliospores is initiated outside the original layer (fig. 3). The apical teliospore elongates greatly and increases in size, while the basal cells apparently grow little or not at all. The teliospore apparently retains its binucleate condition until it is almost mature. Very clear cases of nuclear fusion in the teliospores were observed, and one such case is illustrated in figure 3. The teliospores to the left of this sorus are apparently still immature and consequently still binucleate. In these immature spores the nuclei are relatively small, in certain spores being rather widely separated, while in others they lie side by side. In several of the spores illustrated the two nuclei appear to have just commenced to fuse, forming a dumbbell-shaped structure, although the presence or absence of a separating membrane could not be determined. In the center of the sorus the spores possess a single large nucleus, which apparently has resulted from the fusion

of the two nuclei in the immature teliospore. These repeated obser-
vations would indicate that the spores in the center of the sorus attain
maturity first and that maturity progresses centrifugally in the
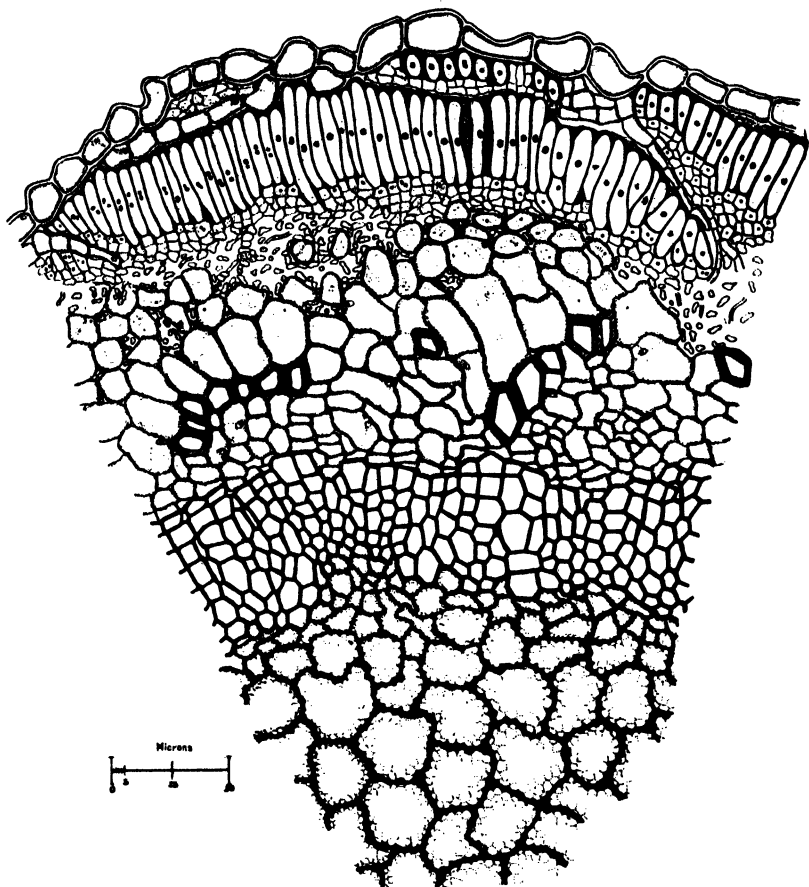
FIGURE 3.—Transverse section of a mature telium in a susceptible host, showing a band
of teliospores embedded in a waxy sorus. On the left of the sorus the teliospores are
still immature and binucleate. The teliospores in the center are mature and possess
a single large fusion nucleus. Note development of secondary layer of teliospores
immediately beneath the still unruptured epidermis. (Camera lucida drawing.)

telium. In this stage of development approximately half of the cor-
tical region of the stem is occupied by the telium and its accompany-
ing hyphae, and eventually the entire stem may become encircled by
a layer of laterally compressed teliospores. The epidermis covering
the telium apparently is not ruptured until the following spring when
the teliospores commence to germinate.

HISTOLOGICAL STUDY OF UREDIAL TYPES

During the summer of 1932 the field reaction to rust of over 100
selections of flax [5] was determined in the flax-rust nursery on peat
soil at Coon Creek, Anoka County, Minn. These observations were

[5] Material obtained from A. C. Dillman, Division of Cereal Crops and Diseases, Bureau
of Plant Industry, U. S. Department of Agriculture.

made as part of another project [6]; hence, the detailed reactions of all of the selections are not given in this paper, although the reactions of the varieties recorded are based on these observations. It is possible that physiologic specialization exists in flax rust and that in other parts of the country the field reactions are not the same as at Coon Creek.

Under field conditions flax may react in five different ways to the flax-rust organism, depending upon the variety concerned. A variety may be immune, highly resistant, resistant, incompletely susceptible, or completely susceptible. The reaction types, erected on the basis of the uredium, have been designated respectively, 0, 1, 2, 3, 4. It was thought that a histological study of the different types of uredia might cast some light upon the problem of varietal resistance to flax rust.

Accordingly, rusted flax material was collected from the field plots, and uredia typical for the above-mentioned reactions were studied histologically. The material was killed in medium chromo-acetic killing solution, dehydrated in the butyl alcohol series, embedded in paraffin, stained with the triple combination of safranine, gentian violet and orange G, and sectioned longitudinally.

Type 0; immune.—Flax varieties immune from rust, such as Rio C. I. 280, Walsh C. I. 645, and Minnesota Selection C. I. 661, in general, show no symptoms whatever of the presence of the flax-rust organism within their tissues.

Type 1; highly resistant.—Flax varieties highly resistant to rust, such as Lino Grande C. I. 381, Bolley Golden C. I. 644, and Indian Type 55, are characterized in their field reaction to rust by the occurrence of necrotic flecks on the surface of infected leaves and by the absence of urediospore formation. When such areas are examined histologically it is found that the host cells in this region are completely broken down and replaced by dark-staining masses of necrotic tissues (fig. 4, *A*). It is rather remarkable that fungus hyphae were not observed among the remnants of the host cells, except at the extreme margins of the necrotic area. There is no indication whatever of the formation of a typical uredium, and all of the host tissue, including the epidermal cells in the vicinity of the infection zone, have apparently been broken down and digested. In addition, the line of demarcation between healthy and necrotic tissue is extremely sharp, which forms a basis for interesting conjecture. In varieties that react to rust in this manner, it seems possible that the fungus establishes parasitic relations with the host cells and destroys the latter so rapidly that it ultimately defeats its own ends by starving, due to its own inability to develop sufficiently fast to keep ahead of the dissolution of the host cells. If such is the case, it is reasonable to suppose that the rust hyphae themselves would ultimately undergo digestion, which would account for their absence in the center of the necrotic areas. If the rust hyphae failed to develop fast enough to keep ahead of the breaking down of the host cells, and thus died from lack of nutriment, one would not expect to find such hyphae in the region bordering the necrotic zone, as actually are found.

A second hypothesis may, therefore, be advanced to explain the mechanics of this type of rust reaction. The rust fungus may suc-

[6] Conducted in cooperation with C. C. Allison at Coon Creek.

ceed in penetrating to the interior of the host and may successfully establish parasitic relations. For a brief period the parasite may derive sufficient nutriment from the association to allow for its limited development, but ultimately a toxin may be produced by the breaking down of the parasitized host cells. Such a toxin might well be injurious to the rust fungus and eventually result in the rust hyphae depending upon their own reserve food material for existence after the supply of available food material from the host had been exhausted. Such a condition would eventually result in the death of the pathogen, and the net result would be the occurrence of an area in the leaf composed of broken-down host tissue with a few isolated rust hyphae at its extremity that had survived
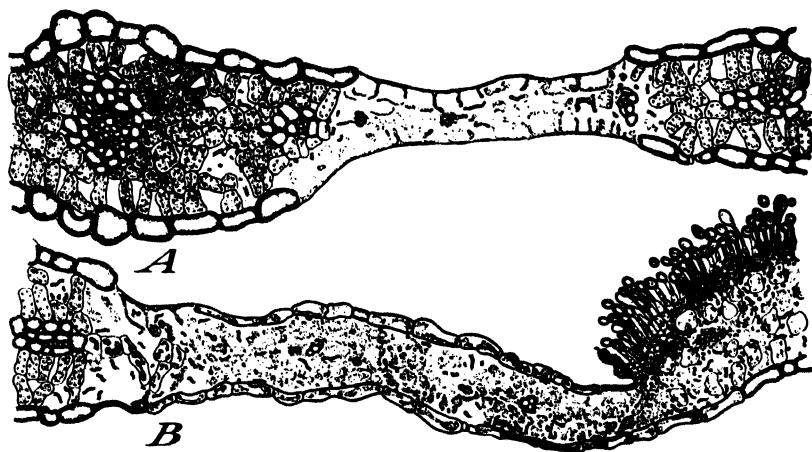
Figure 4.—Uredial pustule types. (Camera lucida drawings.) *A*, Highly resistant, type 1, pustule on Lino Grande flax. Note complete necrosis of host tissue in the infection zone and the absence of urediospore production. *B*, Resistant, type 2, pustule on the flax variety North Dakota Resistant 112; uredial pustule surrounded by a zone of completely broken-down host tissue.

but were incapable of causing further injury to the host. Such a phenomenon would result in the production of a rust reaction similar to that characteristic of type 1.

Type 2; resistant.—Certain varieties of flax, as, for example, Pale Pink (Brine) C. I. 649, Tammes Pale Blue C. I. 333, North Dakota Resistant C. I. 112, and Minnesota Selection C. I. 719 produce type 2 uredia. Externally the type 2 uredium closely resembles the type 1, consisting mainly of a necrotic zone, which, however, upon closer examination is found to possess a small group of urediospores in the center of the necrotic region. When viewed in longitudinal section (fig. 4, *B*) the small uredium is found to be encircled by a fairly wide zone of broken-down host tissue. In this region the mesophyll cells of the host have become transformed into irregular, dark-staining masses, scattered throughout which may be observed hyphal segments of the rust fungus. Contrary to the situation in the highly resistant type 1, the epidermal cells of the host in the type 2 uredium are not destroyed, although they are heavily parasitized and somewhat shrunken; they contain large haustoria of the rust fungus. In the region where the urediospores are produced the leaf is decidedly arched and greatly shrunken as compared with

healthy portions of the leaf. By far the greater part of this type of uredium is composed of dead masses of host cells, and the urediospores usually are quite limited in number and confined to a relatively small area in the center of the necrotic zone.

In this type of uredium a condition similar to that postulated for the type 1 may occur. The rust fungus apparently enters the host and parasitizes the mesophyll tissues of the leaf, upon which it lives and succeeds in developing a rather sparse hyphal system that extends only a relatively short distance through the host leaf system. Ultimately, either through lack of sufficient nutriment, or through the toxic effects of metabolic by-products arising from the breaking down of the host cells, the rust fungus dies, and its zone of activity is thus rather restricted and localized. However, in this type of reaction the rust fungus apparently succeeds in deriving sufficient food material from the association to enable it to produce relatively few urediospores before the delicate symbiotic balance between host and parasite is upset.

Type 3; incompletely susceptible.—A large number of flax varieties, e. g., Ottawa White-flowered C. I. 24, Indian Type 124, and Pale Blue C. I. 387, are characterized in their rust reactions by the development of uredia that are, however, surrounded by a well-defined chlorotic area. A longitudinal section of this type of uredium is illustrated in figure 5, *A*. It will be observed that there is no necrosis, and the fungus has succeeded in making considerable development and has culminated its activity by the production of numerous urediospores. However, in the immediate vicinity of the uredium, below the uredium, and within a narrow zone encircling it, the chloroplasts of the host cells have been destroyed, thus accounting for the chlorotic area surrounding the uredium, which is characteristic of this type of infection. The type 3 uredia are characteristically much smaller than the type 4, but they may break through both leaf surfaces, as illustrated in figure 5, *A*. Except for the smaller size and the presence of the chlorotic ring encircling them, they are in every way similar to the type 4 uredia. In this type of rust reaction, the symbiotic balance between host and parasite is not perfect, as is indicated by the destruction of the chloroplasts of the mesophyll cells. For this reason the reaction has been termed "incompletely susceptible."

Type 4, completely susceptible.—Varieties of flax completely susceptible to rust, such as the variety Winona C. I. 481, are distinguished by the production of large, extensive uredia that lack the chlorotic zone or necrotic tissue characteristic of varieties resistant to the rust. A longitudinal section of this type of uredium is illustrated in figure 5, *B*.

From the foregoing histological studies it is apparent that the relationship between flax rust and plants resistant to its attack is rather complex. It would appear from the indications obtained that the physiological make-up of the different varieties may influence strongly their reaction to rust, as is evidenced by the different types of uredia produced on different varieties. In addition, it is fully realized that it would be desirable to study histologically early stages of infection in varieties resistant to *Melampsora lini*, and studies of this nature have been undertaken.

UREDIOSPORE GERMINATION IN EXTRACTS FROM FLAX VARIETIES DIFFERING IN REACTION TO RUST

In the histological study of the different types of uredia produced on varieties differing in their reaction to rust, indications were obtained pointing to the fact that differences in the physiological properties of the different varieties might be responsible in part for the different rust reactions as expressed by the type of uredium developed. It was thought logical at this point to approach the problem from the physiological aspect of plant-disease resistance.
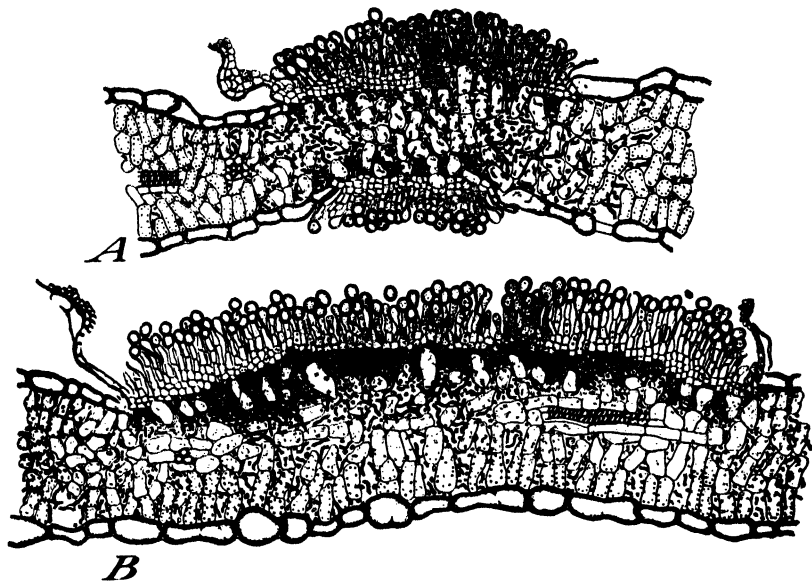
FIGURE 5.—Other uredial pustule types. (Camera lucida drawings.) *A*, Incompletely susceptible, type 3, pustule on Buda flax. Note the area immediately surrounding the pustule in which the host cells are devoid of chloroplasts. *B*, Completely susceptible, type 4, pustule on Winona flax. Note the extensive uredial pustule produced without necrosis or chlorosis of the host cells. The host cells immediately below the pustule still retain their chloroplasts and the rust hyphae ramify extensively throughout the host tissues.

The concept of the physiological nature of disease resistance is founded on the hypothesis that resistance is partly due to differences in the value of different hosts as substrates for the vegetative growth of the pathogen concerned. Ward (*44, 45, 46*), on the basis of investigations concerning the parasitic relationships between the bromegrasses and their rust (*Puccinia dispersa* Eriks. and Henn.), advanced the theory that differences in rust reaction might be due to materials present or produced in resistant plants, but absent in others, that inhibited the growth of the parasite. Leach (*28*) has advanced the theory that materials furnishing nutrients necessary to the development of the stem rust fungus may be present only in susceptible plants and absent in plants resistant to or immune from this fungus. Ezekiel (*13*), from the results of extensive investigations on the nature of physiologic resistance to *Puccinia graminis tritici*, concluded that extracts obtained from different wheat varieties differed in their ability to support the growth of

physiologic forms of stem rust in precise agreement with the respective resistance or susceptibility of the variety to the physiologic forms concerned. Compatibility of physiologic forms with the host varieties was indicated almost universally by greater lengths of urediospore germ tubes in extracts from susceptible varieties, less branching and more infrequent production of apical swellings than in extracts obtained from resistant varieties. Increase in length of the germ tubes was obtained in extracts derived from rusted plants as compared with the germination in extracts obtained from healthy wheat plants. Extracts stored in the refrigerator for 8 months continued to differentiate between forms to which the variety concerned was susceptible or resistant. Extracts prepared by methods involving the application of heat apparently did not contain the material or materials affecting germ-tube elongation.

During the summer of 1933 flax material was collected from healthy flax plants in early stages of maturity growing in the flax-rust nursery at Coon Creek and at University Farm, St. Paul, Minn. A definite amount of each variety was weighed out, and then all parts of the plant—stem, leaves, sepals, and blossoms—were ground up in a meat grinder. The ground-up material was steeped in two and one-half times its own weight of sterile distilled water and placed in the refrigerator for 2 hours. The plant juice was then extracted by squeezing the material through several thicknesses of cheesecloth and then by means of pressure in a hydraulic press equipped with press cups of noncorrosive monel metal. The expressed juice was then filtered twice through filter paper in a Büchner funnel and stored in sterile flasks in the refrigerator at 10° C. The concentration of an extract prepared by this procedure is equivalent to a concentration of 0.4, or a 40-percent solution. In all cases urediospore germination studies were made within 24 hours after the extract was prepared. The 40-percent stock solution was diluted with sterile distilled water to concentrations of 10, 1.0, 0.5, and 0.1 percent. Hanging drops of each extract were made up on sterile cover glasses supported on glass rings contained in a Petri dish lined with moistened filter paper. All glassware used in these experiments was thoroughly cleansed in potassium dichromate-sulphuric acid cleaning solution, washed in alcohol, rinsed in distilled water, and finally sterilized in the hot-air oven at a temperature of 150°. Hanging drops were made up in quintuplicate for each dilution of each extract, and these were inoculated by brushing freshly gathered urediospores on the surface of the drop by means of a stiff camel's-hair brush. Germination counts were made at 12-, 24-, and 36-hour intervals, the individual results given in the following tables being the averages obtained from the five hanging-drop cultures of each extract, that is, of approximately 500 spores. The length of the germ tubes was determined by means of an ocular screw micrometer, the results in tables 1 and 2 being the average of at least 10 determinations for each extract. The length of the longest branch was used as a criterion of length, and the results given were carefully checked by visual observation of the five hanging drops. Check cultures were made up in sterile distilled water. The Petri dishes containing the hanging-drop cultures were incubated in controlled soil-temperature tanks adjusted to run at a temperature of 19°.

TABLE 1.—*Germination of urediospores after 12 and 18 hours in 10-percent extracts from varieties of flax differing in their reaction to rust*

Source of extract [1]	Rust reaction [2]		Germination and length of germ tubes			
	Preva-lence	Type no.	12 hours		18 hours	
			Germi-nation	Length	Germi-nation	Length
	Percent		*Percent*	*Microns*	*Percent*	*Microns*
Distilled water			55	345	55	380
Winona C. I. 481	90	4	42	257	63	328
Bison C. I. 389	1-15	3-4	43	289	50	318
Redwing C. I. 320	15	1	36	291	37	292
Pale Blue C. I. 176	15	1	16	301		
Walsh C. I. 645	0	0	7	173	13	255

[1] Extracts from healthy plants.
[2] Based on Coon Creek observations, 1932.

At the outset a preliminary experiment was made to determine whether or not extracts from different flax varieties varied in their ability to support the vegetative growth of urediospores of *Melampsora lini*. Samples of flax material were collected from healthy plants of Winona, Bison, Redwing, Pale Blue, and Walsh flax growing in the rust nursery at University Farm and were extracted by the methods previously outlined. The stock solutions were diluted with sterile distilled water to a concentration of 10 percent of the natural strength of the plant juice. Hanging drops made up in quintuplicate were inoculated with a bulk sample of urediospores freshly gathered from the field plots and were incubated at 19° C. The experiment was repeated several times to ensure uniformity of technic, observations being made in each case. The experiment was finally set up, freshly prepared extracts from the different varieties being used. The results obtained are given in table 1. These results indicate clearly that the ability of the extracts to support the growth of flax rust urediospores varies in accordance with the resistance or susceptibility of the varieties from which they were obtained. The reduction in percentage germination below the check culture (distilled water) was particularly striking in extracts obtained from the highly resistant variety Pale Blue and the immune variety Walsh. The germ-tube length in extracts obtained from the variety Walsh was considerably reduced when compared with the check culture in sterile distilled water, but the most striking difference was in the nature of the germ tubes in the different extracts.

In sterile distilled water the germ tubes were very irregular and greatly branched (fig. 6, *A*), similar to urediospore germ tubes of *Puccinia graminis tritici* described by Ezekiel (*13*). In extracts obtained from the susceptible variety Winona the germ tubes were strong and regular, and the branching was confined to the end of the germ tube (fig. 6, *B*). In extracts obtained from highly resistant varieties, such as Redwing and Pale Blue, the germ tubes were inclined to be irregular and coiled (fig. 6, *D*). In extracts from the immune variety Walsh the germ tubes were extremely irregular and branched and were inclined to coil rather tightly (fig. 6, *E*).

From the results obtained in this preliminary experiment, it was concluded that there are differences in the ability of plant extracts from flax varieties differing in their field reaction to rust to support the vegetative growth of urediospores of *Melampsora lini.*

A second experiment was conducted to determine the effect of dilution of extracts from different flax varieties on their ability to support the growth of urediospore germ tubes.   Extracts were obtained from several flax varieties as in the previous experiment, except that rusted as well as healthy Winona plants were used for extraction.   The extracts were handled in exactly the same way as in the previous experiment, the stock solutions being diluted
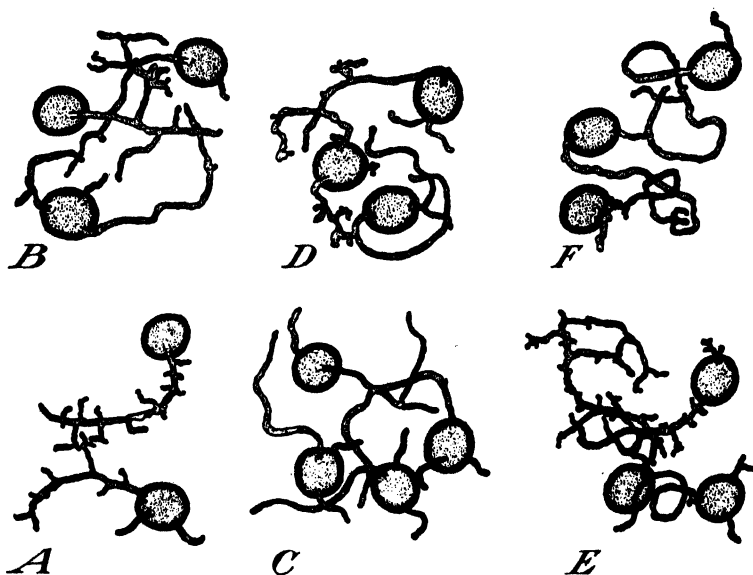
FIGURE 6.—Effect on the germination of urediospores of plant extracts from flax varieties differing in their reaction to rust: *A*, Germination in sterile distilled water; *B*, germination in extracts obtained from healthy plants of the completely susceptible variety Winona; *C*, germination in extracts obtained from rusted plants of the completely susceptible variety Winona; *D*, germination in extracts obtained from the resistant variety Redwing; *E*, germination in extracts obtained from the immune variety Walsh; *F*, germination in extracts obtained from the immune variety Rio.

to concentrations of 5 and 1 percent.   The results of this experiment are given in table 2.   The percentage germination in the different extracts agrees fairly closely with the results obtained in the previous experiment, the different extracts supporting the growth of urediospore germ tubes in accordance with the resistance or susceptibility to rust of the varieties from which they were obtained. No apparent differences appeared in this experiment between the percentage germination of urediospores in extracts obtained from healthy and from rusted Winona plants.   However, the rusted plants from which the latter extract was obtained were only in the initial stages of uredium formation and were not heavily infected.   Differentiation in germ-tube length was much sharper in this experiment than in the previous one.   The length of the germ tubes was exceptionally low, for some reason, in sterile distilled water, but they were very irregular, as previously described.   Germ-

tube length was again considerably reduced in extracts from the immune variety Walsh, the nature of the germ tubes being the same as in the previous experiment (fig. 6, *E*). The nature of germ-tube growth in extracts from rusted Winona plants is illustrated in figure 6, *C*. The concentration of the plant extract appeared to affect germination but little.

TABLE 2.—*Effect on urediospore germination of extracts of different percentage concentrations from varieties of flax differing in their reaction to rust*

1- AND 5-PERCENT CONCENTRATIONS

Source of extract [1]	Rust reaction		Concentration of extract	Germination and length of germ tubes			
	Prevalence	Type no.		12 hours		36 hours	
				Germination	Length	Germination	Length
	Percent		*Percent*	*Percent*	*Microns*	*Percent*	*Microns*
Distilled water............	....	....	....	46	153	54	....
Winona C. I. 481 (rusted)......	90	4	{ 5 / 1	42 / 41	253 / 282	46	....
Winona C. I. 481............	90	4	{ 5 / 1	35 / 40	264 / 286	48	....
Bison C. I. 389............	1–15	3–4	{ 5 / 1	23 / 28	276 / 325	31	....
Redwing C. I. 320............	15	1	{ 5 / 1	37 / 32	200 / 207	37	....
Walsh C. I. 645............	0	0	{ 5 / 1	14 / 14	195 / 173	17	....

1- TO 0.1-PERCENT CONCENTRATIONS

Distilled water............	....	....	....	39	213	....	234
Winona C. I. 481 (rusted)......	90	4	{ 1.0 / .5 / .1	16 / 38 / 34	212 / 357 / 282	16 / 34 / 28	.... / 381 / 278
Winona C. I. 481............	90	4	{ 1.0 / .5 / .1	14 / 19 /	161 / 295 /	...... / 26 /	...... / 300 / ......
Rio C. I. 280............	0	0	{ 1.0 / .5 / .1	13 / 8 / 27	191 / ...... / 338	14 / 20 /	...... / ...... / 332

[1] Extracts from healthy plants except where indicated.

In the previous experiment the results indicated that no differences existed in urediospore germination in 5- and in 1-percent extracts. Another experiment was therefore set up to determine whether extreme dilution of the extracts would affect urediospore germ-tube growth. Extracts were made from the immune variety Rio and from healthy and heavily rusted Winona plants, the procedure of extraction being exactly the same as in previous experiments, the stock solutions being diluted with sterile distilled water to concentrations of 1.0, 0.5, and 0.1 percent of the initial concentration of the plant juice. From the results in table 2 it is apparent that decided differences existed in the germination in different extracts. In extracts obtained from the immune variety Rio, the percentage germination after 12 hours' incubation was considerably reduced as compared with the check series in sterile distilled water. Furthermore, extreme dilution of this extract (0.1 percent) appeared to lessen its inhibitory effect, the germination of 13 percent in the 1-percent

extract as compared with 27 percent in the 0.1-percent extract appearing to be significant. The germination of 8 percent in the 0.5-percent extract is difficult to explain, but was probably due to experimental error. The extract from rusted Winona plants stimulated the percentage germination to a certain degree; the high concentration had an inhibitory effect, the optimum concentration being 0.5 percent. The results of measuring the length of the germ tubes substantiated the percentage germination counts, stronger extracts from the immune variety Rio reducing the germ-tube length, and stimulating it in extracts of 0.1-percent concentration as compared with the check cultures in sterile distilled water. In 0.5 percent extracts obtained from Winona flax plants the germ tubes were significantly longer than in distilled water, germ-tube length being greater in extracts from rusted plants than in extracts from healthy Winona plants. Percentage germination counts and germ-tube lengths were also determined at the end of 24 hours' incubation, and in general the results check reasonably well with the determinations made at the end of 12 hours' incubation. The gaps in the table are due to contamination of the cultures and also to the fact that excessive coiling of the germ tubes in many cases made it impossible to estimate accurately germ-tube length.

The differences in germination in extracts from healthy and rusted Winona plants are inconclusive in view of the negative results obtained in the initial experiment, and further investigations on this point will be necessary.

From the foregoing experiments it was concluded that extracts obtained from flax varieties differing in reaction to rust differ in their ability to support the growth of urediospore germ tubes in accordance with the resistance or susceptibility of the varieties from which they were obtained. Such differences are expressed in the percentage germination, in the length of the germ tubes, and in the nature of the germ tubes in the different extracts.

RELATION OF MORPHOLOGY OF THE HOST TO RESISTANCE

In recent years the nature of morphological resistance to certain diseases has been studied extensively, and the accumulated literature is voluminous. The possible exclusion of parasites by means of unusual thicknesses of cuticle, by the development of layers of corky cells, and by the presence of unusually tough membranes has been suggested independently by several workers as possible factors in determining resistance. Extensive investigations have been made of morphological resistance in cereal rusts, and it has been shown by Hart (*17*) and others that the mycelium of *Puccinia graminis tritici* is limited within the host plant entirely to the chlorenchymatous tissue, the sclerenchymatous tissue remaining impervious to attack. Hart concluded from her studies of the morphology of the peduncle of numerous wheat varieties that wheats generally resistant to stem rust in the field possessed more sclerenchyma and less parenchyma than the highly susceptible varieties. The size, shape, and arrangement of the parenchyma strands in the peduncle proved to be important in determining the spread of the rust mycelium and also in limiting the size of the uredia. The same conclusions were arrived at by Hursh (*22, p. 407*), who states:

In those varieties, therefore, in which there is a large amount of collenchyma, large uredinia are likely to be produced, while in those varieties in which the collenchyma bundles are small, the uredinia are likely to be narrowly linear. Varieties in which there is a great deal of sclerenchyma are likely to be injured less by the rust, as there is a mechanical limitation to the spread of the mycelium.

Thus, in the light of our present knowledge, morphological resistance in wheat to stem rust appears to be due to the large amount of sclerenchyma in proportion to the amount of parenchyma present in the leaf, stem, or peduncle of the variety concerned.

MORPHOLOGY OF THE FLAX LEAF

The morphology of the flax leaf was studied by sectioning and staining leaves of several varieties of flax. The epidermis of the flax leaf is composed of irregularly rectangular cells, the cells of the lower epidermis being in most cases considerably larger than those of the upper epidermis (fig. 1, *B*). There is a rather indefinite palisade layer of loosely arranged, rectangular cells situated immediately beneath the epidermis. The mesophyll consists of loosely arranged, approximately spherical cells containing numerous chloroplasts around their periphery. The leaves of most flax varieties have a large vascular bundle associated with the somewhat prominent midrib, the other smaller vascular bundles lying parallel to the median one. Usually associated with the vascular bundles are a number of small sclerenchyma cells, but the formation of large sclerenchyma girders, as reported for wheat, was not observed in the leaves of any of the varieties of flax examined. The leaves of numerous flax varieties, differing widely in their reaction to rust in the field, were carefully examined histologically, but no striking major anatomical differences were observed, although minor variations, such as differences in the size and shape of the epidermal cells, were noted.

From these observations it was concluded that there are no major anatomical features of the flax leaf which could explain the various degrees of resistance and susceptibility of the different varieties to rust.

MORPHOLOGY OF THE FLAX STEM

For a study of the structure of the flax stem, several varieties of flax were grown in pots in the greenhouse and material was sectioned immediately prior to the flowering stage. For detailed morphological studies material was killed in Flemming's weaker solution (*8*), dehydrated in butyl alcohol (*49*), embedded, and stained with Flemming's triple stain (*48*). The butyl alcohol method of dehydrating was found to be superior to the ordinary alcohol-xylol series, causing less plasmolysis of the cells, producing less hardening of the stem tissues, and saving a great deal of time. Numerous staining combinations were used, but the triple combination of safranine, gentian violet, and orange G was most satisfactory. In addition, fresh material was sectioned freehand and stained with lactophenol cotton-blue stain (*30*). All measurements of epidermal cells were made from this material.

The flax stem is a hollow cylinder. It has an epidermis of varying thickness, composed of relatively thick-walled cells, which may

be rectangular or isodiametric depending upon the variety. Immediately beneath the epidermis is the cortical parenchyma, which varies in extent in different varieties and is composed of irregular cells arranged rather loosely. The outer cells of the cortex are usually chlorenchymatous and are limited internally by rather large, fairly regular, compactly arranged parenchyma cells which surround the cortical fibers. The vascular tissues in most of the varieties examined are arranged in a rather extensive continuous ring, but in some varieties they appear as isolated bundles. Internal to the vascular tissue is the inner parenchyma, comprising large regular cells surrounding the pith cavity.

From a study of the anatomy of the stems of various flax varieties certain generalized comparisons may be drawn between susceptible, resistant, and immune varieties. In the susceptible varieties examined, such as Crepitans and Winona, the epidermal cells in general are rectangular and fairly thin-walled, and there is no indication of extensive cuticular development (fig. 7, *A*). Many of the susceptible varieties possess a continuous ring of large, thick-walled cortical fibers, but the size and distribution of the cortical fibers do not appear to be consistently of this type in all susceptible varieties. In the variety Pinnacle, which is slightly less susceptible than Crepitans, the epidermal cells tend to approach the isodiametric condition, are slightly thicker-walled, and possess a visible but very thin cuticle (fig. 7, *B*). In such a variety there is also a tendency to develop a single layer of small, regular cells immediately below the epidermis. Observations indicate that this layer of cells comprises a collenchymatous hypodermis. In the variety Pinnacle this layer of cells is not continuous.

Resistant varieties, such as Bolley Golden C. I. 644 (fig. 7, *C*), Lino Grande C. I. 381, and Abyssinian C. I. 300, are characterized by large, almost isodiametric, thick-walled epidermal cells. The outer epidermal wall in most cases is much thicker than the inner cell wall, and in the resistant flax varieties examined it was covered with a fairly thick, well-defined cuticle. In these varieties also, immediately underlying the epidermis, a continuous layer of collenchymatous hypodermis one cell thick was present.

In immune varieties, such as Minnesota Selection C. I. 661 (fig. 7, *D*), Rio C. I. 280, Indian Type 46, and Walsh C. I. 645, the epidermal cells appear to be completely isodiametric, with a thick outer wall and a very thick cuticle, which in the variety Rio sometimes attains a thickness of 2μ to 4μ.

From the foregoing study of the morphology of the flax stem it is apparent that there are certain morphological differences between different varieties of flax, and it would seem likely that these differences may operate in determining resistance or susceptibility to flax rust, probably in conjunction with other factors.

THICKNESS OF THE EPIDERMIS IN RELATION TO THE FORMATION OF UREDIA

It is known from the investigations of Hart (*17*) that the size and shape of the epidermal cells, the thickness of the outer epidermal wall, and the presence of a cuticle play an important role in pustule formation in the cereal rusts. In varieties of flax susceptible to flax rust, such as Winona, the fungus apparently encounters little difficulty

in rupturing the epidermis, as numerous large uredia develop. In resistant and partially resistant varieties, such as Buda, Lino Grande, and Bolley Golden, on the other hand, the uredia are generally smaller and fewer; and in some cases on the leaves the symptoms of
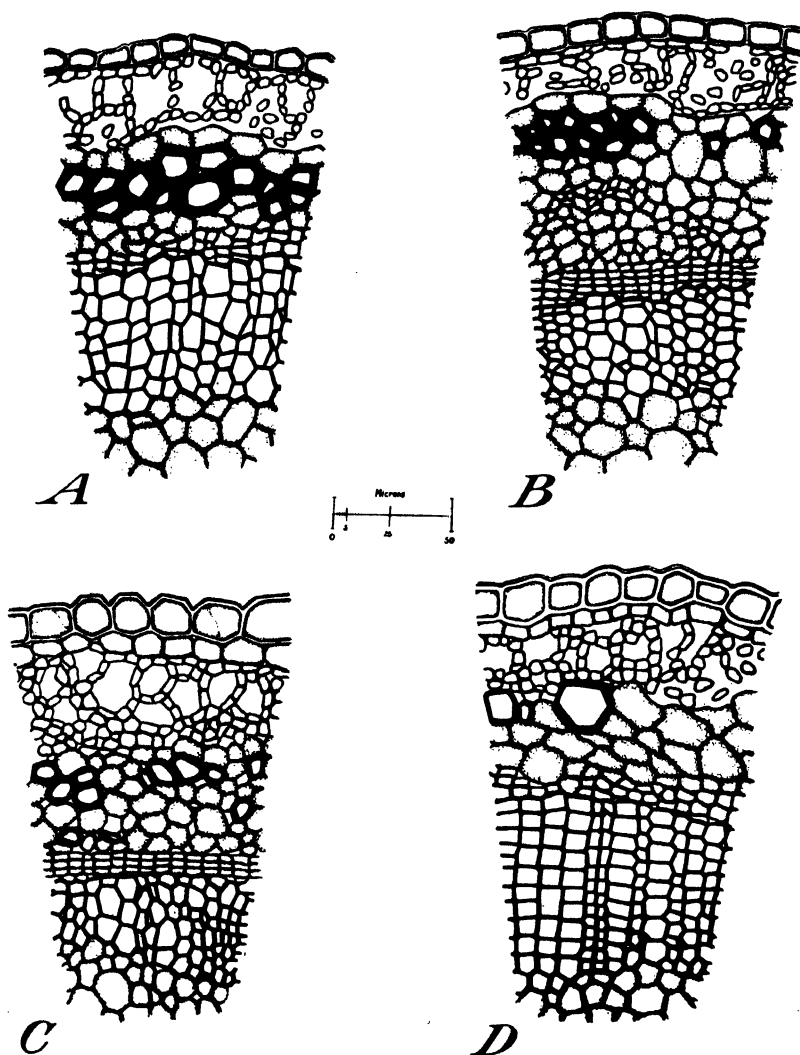
FIGURE 7.—Stem structure in flax varieties differing in their field reaction to rust. (Camera lucida drawings.) *A*, Variety Crepitans, completely susceptible; *B*, variety Pinnacle, partially resistant; *C*, variety Bolley Golden, resistant; *D*, variety Minnesota Selection C. I. 661, immune.

infection are confined to flecks on the upper leaf surface. In some cases, subepidermal pustules are developed on the stems of certain resistant varieties, apparently as a result of the rigidity of the epidermal membrane.

An analogous situation has been noted in wheat in connection with stem rust. Hart (*17*) observed that the epidermal membranes of

wheat varieties susceptible to stem rust (Little Club, Ruby, and Marquis) were comparatively thin and easily ruptured. The epidermis of some wheat varieties fairly resistant to stem rust (Khapli, Kota, and Webster), on the other hand, was found to be very thick as compared with that of the susceptible varieties, and in these varieties subepidermal pustules are commonly developed.

In this investigation, the thickness of the epidermal membrane of several flax varieties was determined by sectioning freehand fresh material, staining with lactophenol, cotton-blue stain, and then measuring with an ocular screw micrometer. The results, which are given in table 3, should be considered as relative and not in any way as absolute, for the thickness of the epidermal membrane undoubtedly varies considerably under different environmental conditions.

The immune varieties examined were found to have a very thick cuticle and a thick epidermal membrane. The individual cells of the epidermis were almost perfectly isodiametric, the ratio of length to width varying from 1.0:0.92 to 1.0:1.01. Resistant varieties had a slightly thinner epidermal membrane than the immune varieties examined, and they also had a thick cuticle. The epidermal cells were almost isodiametric, the ratio of length to width being about 1.0:0.8. Susceptible varieties possessed a relatively thin epidermis, a well-developed cuticle generally was absent, and the epidermal cells were elongate-rectangular, the ratio of length to width varying from 1.0:0.44 to 1.0:0.65. The results of these determinations would appear to indicate that the thickness of the epidermal membrane may play an important role in pustule formation.

TABLE 3.—*Dimensions of the epidermal cells of varieties of flax differing in their reaction to rust*

Reaction and variety [1]	Rust reaction		Length of epidermal cells	Width of epidermal cells	Ratio length to width	Cuticle
	Prevalence	Type no.				
Immune:	*Percent*		*Microns*	*Microns*		
Rio C. I. 280	0	0	22.9	23.2	1:1.01	Very thick.
Minnesota Selection C. I. 661	0	0	27.2	18.3	1:.67	Fairly thick.
Minnesota Selection C. I. 651	0	0	22.3	20.5	1:.92	Very thick.
Indian Type 46	0	0	20.8	19.2	1:.93	Thick.
Resistant:						
Lino Grande C. I. 381	Tr.	1	25.6	17.0	1:.66	Do.
M–25–221 C. I. 423	45	1	28.2	23.6	1:.83	Fairly thick.
Bolley Golden C. I. 644	50	1	22.5	17.7	1:.78	Do.
Indian Type 55	50	1	19.3	16.6	1:.86	Very thick.
Abyssinian C. I. 300	55	1	19.4	19.3	1:.99	Do.
Susceptible:						
Buda C. I. 326	85	3	24.4	15.9	1:.65	Thin.
Pinnacle C. I. 693	60	4	20.8	12.3	1:.59	Absent.
Crepitans C. I. 506	70	4	20.2	8.9	1:.44	Do.
Winona C. I. 481	90	4	27.0	15.2	1:.56	Do.
Deep Pink C. I. 648	90	4	22.8	15.6	1:.68	Very thin.
Indian Type 22	100	4	25.5	17.1	1:.67	Do.

[1] C. I.=Accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

RESISTANCE OF THE EPIDERMIS TO PUNCTURE

The ability of *Melampsora lini* to develop normal uredia varies greatly in the different varieties of flax. In completely susceptible varieties, such as Winona, characteristic type 4 uredia usually break through both leaf surfaces. In partially susceptible varieties, such as

Indian Type 12, Indian Type 55, and Lino Grande, a few well-developed uredia may occur on the lower leaf surface, but often there are none, and numerous hypersensitive flecks indicating resistance are present. It would thus seem reasonable to suggest that the tensile strength of the epidermal membrane may play some part in determining the ability of the fungus to form normal uredia on different varieties. Accordingly, the resistance of the epidermis of a number of varieties of flax to puncture was determined by means of a modified Joly balance.

The Joly balance was first adapted to the study of the resistance of various plant tissues to puncture by Hawkins and Harvey (*18*), the apparatus later being modified further by Melander and Craigie (*34*). As modified by the latter workers, the apparatus consisted of the ordinary Joly balance standard, but the pan of the balance was removed and replaced with a coupling designed to carry a carefully ground phonograph needle. The downward path of the needle was maintained in a vertical direction by means of a wire guide attached to the standard of the apparatus. In making a determination, the leaf material was placed on a paraffin block on the stage of the apparatus and weighted down by means of a lead weight provided with a slit to allow for the insertion of the needle. The needle was then brought to rest on the surface of the leaf material, and an indicator on the needle coupling was so adjusted as to correspond with a hair line etched on a small mirror placed immediately behind the needle coupling. The reading on the vernier scale was then recorded, and by decreasing the tension on the spring and closely observing the needle indicator through a magnifying glass, it was possible to observe the exact moment of puncture of the leaf epidermis, a perceptible drop in the needle being observed distinctly when this occurred. The reading on the vernier scale was again recorded, and the difference between the initial and final readings gave the distance it was necessary to lower the needle in order to effect penetration of the leaf surface. A fall of 1 cm on the vernier scale was found by experimentation to be equivalent to a pressure of 502 mg. The diameter of the needle used was approximately 25μ. Determinations were made for the upper and lower leaf surface—10 leaves being used for each variety—and 50 determinations were made for each leaf surface. To ensure uniform experimental conditions, one-half of the determinations on each variety were made in the morning and one-half in the afternoon, and approximately similar days were selected during the course of the work, which was done in the greenhouse. To ensure uniform leaf turgor, fresh leaves were removed from growing plants of the same age, and the determinations for each leaf surface were made immediately. As a rule, only five determinations were possible for each surface of a single leaf, as the leaf wilted soon after it was removed from the plant; the resistance of wilted leaves to puncture probably would be different from that of turgid leaves.

The results of the determinations are given in table 4. It is apparent that the epidermis of the susceptible varieties examined was far easier to puncture than that of resistant varieties. The leaf epidermis of the resistant varieties was in turn harder to puncture than that of immune varieties. In immune varieties, ap-

parently, the fungus fails to establish successful parasitic relations, whereas in resistant varieties it does appear to establish itself for a while within the tissues of the host, but the uredia subsequently developed are restricted in size and in some cases appear only on the lower leaf surface. The results presented in table 4 show a significant difference in the amount of pressure required to puncture the lower and upper leaf epidermis of resistant varieties, the upper epidermis being consistently more resistant to puncture, and this may account for the absence of uredia and the appearance of flecks on the upper leaf surface of the resistant varieties examined.

TABLE 4.—*Pressure required to puncture the outer epidermal wall of the upper and lower leaf surfaces of varieties of flax differing in their reaction to rust (as measured by the July balance)*

Reaction and variety	Leaf surface	Range	Mean	Standard deviation	Mean differences and probable errors of the differences between the two means
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
Immune:					
Minnesota Selection C. I. 664.	Upper	572.28– 853.40	683.02± 7.45	78.10± 5.27	} 41.78±10.81
	Lower	512.04– 903.60	641.24± 7.83	82.13± 5.54	
Minnesota Selection C. I. 661.	Upper	602.40– 853.40	723.90± 8.16	85.55± 6.07	} 103.93±14.05
	Lower	351.40– 903.60	619.97±11.44	120.00± 8.10	
Indian Type 46	Upper	486.94–1,099.38	711.44±11.06	116.00± 7.82	} 78.94±14.32
	Lower	476.90– 903.60	632.50± 9.10	95.60± 6.50	
Resistant:					
Indian Type 12	Upper	476.90–1,014.04	708.22±11.68	132.90± 8.96	} 76.79±15.57
	Lower	426.70– 858.50	631.43±10.30	108.00± 7.28	
Lino Grande C. I. 381	Upper	602.40–1,280.10	988.54±15.92	166.60±11.32	} 123.44±20.27
	Lower	517.06–1,305.20	865.10±12.55	186.10±17.75	
Buda C. I. 326	Upper	581.34–1,275.08	901.43±13.66	143.20± 9.65	} 52.15±19.05
	Lower	597.38–1,104.40	849.28±13.28	138.60± 9.35	
Susceptible:					
Winona C. I. 481	Upper	416.66– 858.42	606.22± 8.84	92.74± 6.25	} 87.45±11.70
	Lower	366.46– 677.70	518.77± 7.70	78.05± 5.27	
Indian Type 22	Upper	376.70– 702.80	518.49± 8.84	92.72± 6.25	} 31.97±13.13
	Lower	301.20– 778.10	486.52± 9.71	102.00± 6.88	
Deep Pink C. I. 648	Upper	351.40– 798.19	519.76± 9.74	102.00± 6.88	} 89.85±11.86
	Lower	291.16– 552.20	429.91± 6.78	71.05± 4.79	
Crepitans C. I. 506	Upper	281.12– 677.70	463.85± 7.36	77.01± 5.12	} 35.04± 9.93
	Lower	266.06– 602.40	428.81± 6.67	59.80± 4.70	

A similar experiment was made to ascertain the pressure required to puncture the epidermis of stems of the same varieties and plants used in determining the pressure required to puncture the leaf epidermis. The results are given in table 5. It is evident that the stems of the susceptible varieties examined were far easier to puncture than those of the resistant and immune varieties.

TABLE 5.—*Pressure required to puncture the outer epidermal wall of stems of varieties of flax differing in their reaction to rust*

Reaction and variety	Range	Mean	Standard deviation
	Milligrams	*Milligrams*	*Milligrams*
Immune:			
Minnesota Selection C. I. 664	913.64–1,346.36	1,142.14± 9.78	102.5 ±6.91
Minnesota Selection C. I. 661	978.90–1,286.12	1,148.40± 7.50	78.8 ±4.37
Indian Type 46	993.96–1,321.26	1,153.03± 7.90	82.8 ±5.59
Resistant:			
Indian Type 12	848.38–1,249.98	1,078.50± 8.73	91.4 ±6.17
Lino Grande C. I. 381	908.62–1,301.18	1,099.86± 8.77	90.9 ±6.13
Buda C. I. 326	828.30–1,104.00	954.54± 8.70	91.1 ±6.14
Susceptible:			
Winona C. I. 481	702.80–1,109.42	872.76±10.00	104.8 ±7.07
Indian Type 22	652.60– 933.72	804.04± 6.64	69.71±6.64
Deep Pink C. I. 648	502.00– 853.40	651.39± 8.90	93.5 ±6.31
Crepitans C. I. 506	692.76– 888.54	789.14± 5.60	58.6 ±3.96

The results of all the foregoing determinations are summarized diagrammatically in figure 8, from which it is evident that there is a definite correlation, in the varieties examined, between rust reaction and the resistance of the epidermal membrane to puncture. The leaves of resistant varieties were found to be more resistant to puncture than those of immune varieties, while those of immune varieties were more resistant to puncture than the leaves of susceptible varieties, a mathematically significant difference existing in nearly every instance between the pressures required to puncture the upper and lower leaf surfaces. The stem epidermis of the resistant varieties examined was found to be more resistant to puncture than
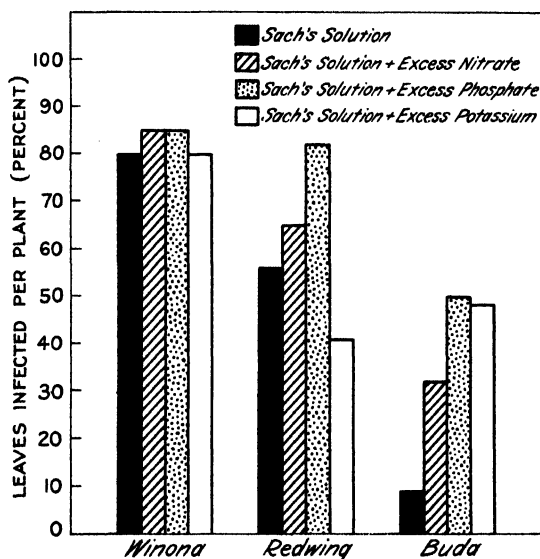
FIGURE 8.—Diagrammatic summary of the pressures required to puncture the leaf and stem epidermis of flax varieties differing widely in their reaction to flax rust, as determined by means of the modified Joly balance.

that of susceptible varieties, very little difference existing between resistant and immune varieties, although the latter required a slightly greater pressure to effect puncture.

The differences in the pressure required to puncture the leaf and stem epidermis of the different varieties of flax examined would indicate that the toughness of the epidermal membrane may play a role in determining or limiting formation of uredia in varieties of cultivated flax differing widely in their reactions to *Melampsora lini*.

Melander and Craigie (*34*), using the same methods as were employed in this investigation, obtained results indicating that those species of *Berberis* that possessed leaves very resistant to puncture were usually resistant to *Puccinia graminis* also. In addition, leaves of certain resistant species were found to have a very thick cuticle. Resistance of barberry leaves to puncture was found to increase with age of the plant, and this in turn was correlated with increased resistance to infection by stem rust.

Hawkins and Harvey (*18*), using a modified form of Joly balance, found that more pressure was required to puncture the tissues of a variety of potato comparatively resistant to leak, caused by *Pythium debaryanum* Hesse, than was required to puncture the tissues of two susceptible varieties. Correlated with this resistance to puncture is a resistance to infection by *P. debaryanum*.

Leach (*29*), studying the parasitism of *Colletotrichum lindemuthianum*, found that the inner cell wall of susceptible host tissues offers a certain amount of resistance to the growth of the mycelium of the anthracnose organism, retarding apical growth and causing the mycelium to become greatly enlarged and to bend outward before penetration of the cell wall is effected. In old tissues of susceptible varieties of beans, the mycelium is retarded in its development by the increased resistance of the cell walls to penetration. Such retarded mycelium disintegrated, killing the host protoplast, and staining the host cell wall and its contents reddish brown. Growth of the mycelium and penetration of cell walls was studied in sections of living material. The fungus was observed to penetrate through numerous cells without killing the protoplast of the host. The swelling and bending of the hyphae during cell-wall penetration would indicate that mechanical pressure is an important factor in early stages of development of *C. lindemuthianum* in the tissues of the bean.

An interesting analogy may be drawn between the results obtained in the present investigation and those cited above. In the case of the barberry, the presence of a thick cuticle and the resistance of the epidermal membrane to puncture offers an effective barrier to penetration by the sporidial germ tubes of *Puccinia graminis*. Thus, in this example, the resistance of the epidermal membrane prevents the pathogen from entering the host plant, and in certain cases is directly responsible for the resistance of some species of *Berberis* to infection.

In the case of potatoes resistant to *Pythium debaryanum* and beans resistant to *Colletotrichum lindemuthianum*, the degree of the resistance of the cell walls to penetration apparently determines the rate of development of the causal organisms after they have gained entrance to their respective hosts.

In the case of flax rust the toughness of the epidermal membrane in certain flax varieties prevents normal formation of uredia, as the force exerted by the growing uredium is apparently insufficient to rupture the covering epidermal membrane, resulting in the presence of subepidermal uredia and the failure of the urediospores to be liberated. Such a condition may be of considerable significance under field conditions. It is possible that the epidermis of these varieties may prevent normal penetration by sporidial germ tubes, but this phase of the problem was not studied.

OCCURRENCE AND ARRANGEMENT OF THE CORTICAL FIBERS

The occurrence and arrangement of the cortical fibers varies greatly in different flax varieties, and probably also under different environmental conditions. In some varieties they occur as irregular, thin-

walled, isolated, or grouped fibers not forming a continuous ring; in others they occur as regular, thick-walled fibers forming a continuous ring in the cortex of the stem. Cross sections of the cortical fibers in several varieties of flax are shown in figure 9.

It will be noticed that in the immune variety Rio (fig. 9, E) the cortical fibers are very thick-walled, somewhat square in cross section, and form a continuous band of cells in the cortex of the stem, whereas in another immune variety, Indian Type 46 (fig. 9, A), the fibers are very small and occur in isolated groups.

In the resistant variety Lino Grande (fig. 9, F), the fibers are small, thin-walled, irregular, and arranged in scattered groups 1
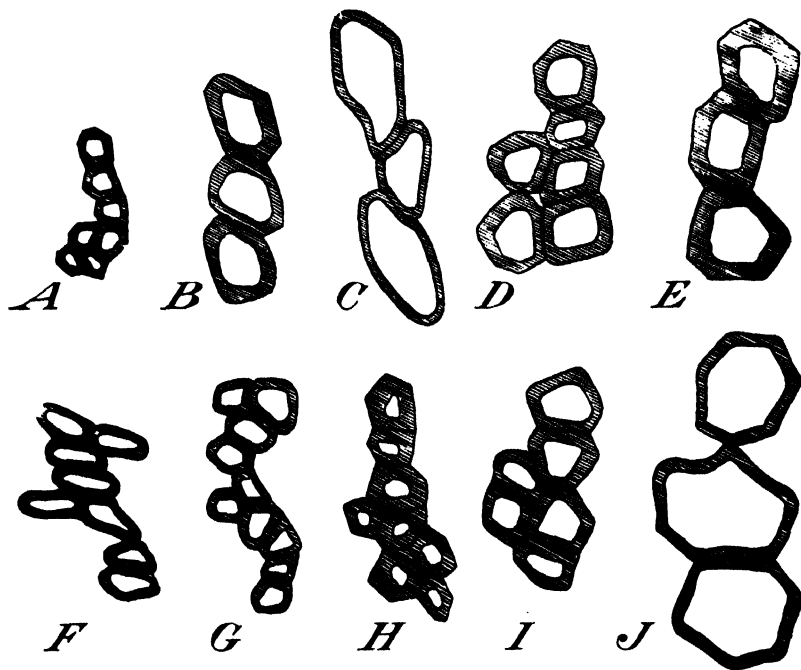
FIGURE 9.—Cortical fiber types in different varieties of flax: *A*, Indian Type 46; *B*, M–25–221; *C*, Deep Pink; *D*, Crepitans; *E*, Rio; *F*, Lino Grande; *G*, Bolley Golden; *H*, Pinnacle; *I*, Winona; *J*, Buda.

to 3 cells deep. In the variety M–25–221 (fig. 9, B), also resistant to rust, the fibers are fairly large, thick-walled, somewhat square in cross section, and arranged in a discontinuous ring in the cortex of the stem. In the resistant variety Bolley Golden, (fig. 9, G), the fibers are small, thin-walled, and occur in small groups scattered irregularly throughout the cortex. In the susceptible varieties there are extreme variations in the size, shape, and arrangement of the fibers. In Deep Pink and Buda they are large, rectangular in cross section, thin-walled, and scattered. In Winona, Crepitans, and Pinnacle (fig. 9, I, D, H), on the other hand, they are thick-walled, tend to be square in cross section, and form a more or less continuous band in the cortex. In these varieties the cortical fibers are par-

ticularly thick-walled and form a well-developed, continuous band of cells 1 to 3 cells deep.

From these observations it is obvious that the occurrence and arrangement of the cortical fibers in flax cannot be connected in any way with the morphological resistance of varieties to rust.

TO WHAT EXTENT DOES THE MORPHOLOGY OF THE HOST OPERATE IN THE RESISTANCE OF FLAX TO RUST?

From the results of the foregoing studies, it is apparent that there are no gross anatomical features comparable to the sclerenchyma girders in the peduncle of the wheat plant which might be a factor in determining the resistance or susceptibility of flax to rust. The fact that the leaf and stem epidermis of certain varieties resistant to rust in the field offers far more resistance to puncture than does the epidermis of certain susceptible varieties may be of considerable importance. Such varieties possessing an epidermal membrane capable of withstanding relatively high strains and thus rupturing only after considerable force has been exerted have been shown to be resistant to rust in the field. While most of these varieties usually produce the characteristic type 1 reaction, with the appearance of flecks and no urediospores on the leaves, a few small scattered uredia occasionally occur on the leaves of these varieties. If one examines such leaves, well-developed uredia containing numerous urediospores will often be observed immediately beneath the epidermis. The urediospores are pressed tightly against the epidermal cells; consequently they are much flattened at the ends and molded to the contours of the epidermal cells, indicating that they have been subjected to considerable pressure exerted by the stromatic mass upon which they were borne. Obviously, the urediospores produced under such conditions may never be liberated from the host and dispersed. Under field conditions this probably would result in a significant decrease in the amount of inoculum liberated in the course of the summer and a consequent reduction in the prevalence of rust.

Correlated with the resistance of the epidermal membrane to puncture is a shortening and thickening of the individual epidermal cells. In most of the varieties with epidermal membranes relatively resistant to puncture the epidermal cells were short and isodiametric, thus accounting, in part, for the amount of pressure required to effect puncture.

It must be admitted that the resistance of a membrane to puncture from without, as determined by the modified Joly balance, may not be a true indication of its resistance to rupture from within. Nevertheless, it is logical to suppose that such determinations do give an indication of the resistance of the epidermal membrane to rupture, and, in fact, the methods used in this work appear to be the only practical means of studying this question.

In addition to having membranes resistant to puncture, the epidermal cells of certain resistant and immune varieties had extremely thick cuticles. Furthermore, the stems of such varieties invariably were provided with a more or less continuous layer of hypodermis immediately below the epidermis.

The fact should not be overlooked that the presence of a thick epidermal membrane, the occurrence of a thick cuticle, and the presence of a hypodermis in some resistant and immune varieties may prevent penetration of the sporidial germ tubes of *Melampsora lini*, as is the case with *Berberis* spp. and *Puccinia graminis*. If this is true, such resistant and immune varieties would escape infection by sporidia of the rust fungus, provided the sporidial germ tubes were unable to penetrate the thick membrane; and, if infection subsequently did occur, it would have to be through the medium of urediospore germ tubes entering through the stomata. Such a condition would obviously result in a significant reduction in the amount of available inoculum in the early summer, and, in addition, the varieties which escaped sporidial infection would not be exposed to infection by urediospores until relatively mature, which might be a factor to be considered. This probability has not been investigated, but it appears to be worthy of careful consideration.

It is apparent that certain morphological features of the flax plant do operate in the resistance of some varieties to rust in the manner outlined above. While these factors alone may not be sufficient to confer resistance, they probably contribute to the resistance of some varieties.

RELATION OF STOMATA TO RUST RESISTANCE

In recent years the "functional" type of resistance has been suggested as a possible factor in resistance to certain diseases. According to the functional resistance hypothesis, the number, size, and movement of the stomata of the host plant are considered in certain cases to contribute toward its resistance to those diseases the causal organisms of which gain entrance to the suscept by way of the stomata. In the case of the rusts the urediospore is liberated, and if it eventually reaches a suitable infection court it proceeds to germinate, provided environmental conditions are favorable. The germ tube continues to grow until it reaches an open stoma, through which it sends an infection peg into the interior of the host, thus initiating infection. Recent investigations have shown that in cereals, and in fact in most plants, there is a regular diurnal movement of stomata, which varies under conditions of a fluctuating environment (*33*). In addition, stomatal movement in wheats has been found to vary in different varieties within the species. Such variations seem sometimes partially to determine the resistance or susceptibility of the variety to stem rust. Thus, the stomata of certain highly susceptible varieties, such as Little Club, Reward, and Quality, open almost immediately at sunrise and remain open during the hours of sunlight, thus allowing ample opportunity for the entrance of urediospore germ tubes. Certain highly resistant varieties, like Webster, Hope, and Velvet Don, on the other hand, do not open their stomata until the dew has disappeared from the leaves, and thus the urediospore germ tubes, in the absence of adequate moisture, fail to extend sufficiently to enter through a stoma when the stoma does open (*17*). Such varieties, by virtue of their stomatal movement, may escape

infection to some extent and are said to possess "functional resist-
ance" to stem rust. It was thought possible that certain flax varieties
might have a functional resistance to *Melampsora lini;* therefore, the
following experiments were made.

PREVIOUS WORK

Pool and McKay (*40*), studying the relation of stomatal move-
ment of *Beta vulgaris* to infection by *Cercospora beticola* Sacc.,
found that germ tubes penetrated sugar beet leaves only through
open stomata. They found also that stomatal movement was in-
fluenced by leaf maturity and by certain environmental conditions.
In mature leaves, stomatal movements were more active than in
young heart leaves, while only very slight movements were observed
in old leaves. The results of inoculations established the fact that
heart and very young leaves were not susceptible to *C. beticola*, young
mature leaves were only slightly so, and mature leaves were quite
susceptible. At daily temperatures of 70° to 90° F., with a relative
humidity of about 60 percent, the stomata on the mature leaves re-
mained open throughout the day. In certain of the experiments
very long germ tubes were produced, but in no case were they ob-
served to penetrate through closed stomata, penetration taking place
only through open stomata.

Caldwell and Stone (*7*), by a method of stripping the epidermis
from wheat seedling leaves inoculated with *Puccinia triticina* Eriks.
and by fixing and mounting the strips in absolute alcohol, were able
to study directly the relation of stomatal aperture at the time of
leaf penetration to the entrance of rust into the host. Closed stomata
were found to offer no barrier to the penetration of wheat seedlings
by urediospore germ tubes of the rust; the studies indicated rather
that the formation of an appressorium over an open stoma stimu-
lated it to close tightly prior to penetration by the rust. Occa-
sionally a small stomatal slit was evident between the appressorium
and the substomatal vesicle, apparently resulting from the penetra-
tion tube pushing between the guard cells of the stoma.

Hart (*17*) found that germ tubes of *Puccinia graminis tritici* enter
wheat only through open stomata, for apparently the urediospore
germ tubes are incapable of forcing their way between the guard cells
when the stomata are closed. She concluded that the uredial stage of
P. graminis tritici causes infection only when there is sufficient mois-
ture for spore germination, when favorable temperatures for uredio-
spore germination prevail, and while the stomata are sufficiently open
to permit the entrance of the germ tubes. Allen (*1*), studying *P.
triticina* on Little Club wheat, found that in material fixed in the
early morning, germ tubes had just entered the host plant and formed
substomatal vesicles when examined histologically. On material fixed
in the afternoon most of the substomatal vesicles had sent out infec-
tion hyphae. She suggests that the time of entrance of *P. triticina*
may be conditioned by the daily stomatal movements of the host and
that entrance depends upon the natural opening of the stomata rather
than upon mechanical force or chemical action.

Hart (*17*), from extensive investigations of the relation of stomatal movements in wheat to resistance to stem rust, concluded: "Certain wheat varieties may escape stem-rust infection because of their characteristic stomatal behavior. Such varieties are said to possess functional resistance to stem rust under certain conditions."

NUMBER OF STOMATA ON LEAVES OF DIFFERENT VARIETIES OF FLAX

Since the urediospore germ tubes of *Melampsora lini* enter the host through stomata, it would be reasonable to suppose that those varieties with the greatest number of stomata per unit area of leaf surface might be the most susceptible to rust, other factors being the same. It therefore seemed desirable to determine the number of stomata on the leaves of flax varieties that differed widely in their reaction to rust.

Several varieties were grown in the greenhouse and the number of stomata on seedling and mature leaves determined. The leaves were removed from the plants and placed immediately in acetoalcohol until clear and then stained in lactophenol, cotton-blue stain. Using the lower power objective of the microscope, the number of stomata on the upper and lower leaf surfaces within a leaf area of 0.5 mm^2 was determined by placing within the ocular of the microscope a cover slip bearing a measured area corresponding to 0.5 mm^2 of leaf surface and counting the number of stomata within that area. The number of stomata within a definite leaf area was determined for the apex, middle, and base of the upper and lower leaf surfaces of each leaf. Several leaves of each variety were used, and 10 microscopic fields were examined for each of the above-mentioned portions of the leaf. The figures given in table 6 thus each represent the average of 10 determinations, the average number of stomata for both surfaces being the average of 60 determinations. The rust reactions of the varieties were recorded during the summer of 1932 in the flax rust nursery at Coon Creek. From the results given in table 6 it will be noticed that the seedling leaves of varieties susceptible to *Melampsora lini* consistently had a larger number of stomata per square millimeter of leaf surface than those of immune varieties. The differences appear to be greater than those between susceptible and resistant varieties. No significant differences are apparent in the number on the upper and lower leaf surface. In some susceptible varieties the apex of the seedling leaves appears to have a greater number of stomata than the middle or basal portions. It seems significant that field observations indicate that infection and subsequent uredium formation appear first at the apex of the leaves. The differences in stomatal number of the various portions of the leaves are more marked in mature leaves (table 6), and the presence of a greater number of stomata at the apex might account for the appearance of the first-formed uredia at the apices. Comparable results were obtained when the stomatal number for mature leaves was determined (table 6). The leaves of susceptible varieties again possessed a slightly greater number of stomata than the immune varieties, the difference between susceptible and resistant varieties being less marked.

TABLE 6.—*Number of stomata on seedling and on manure leaves of varieties of flax differing in their reaction to rust*

(Each figure representing the average of 10 microscopic fields, each containing an area equivalent to 0. mm² of leaf surface)

SEEDLING LEAVES

Reaction, variety, and surface	Average leaf dimensions		Average stomata in 0.5 mm² of leaf surface				Average stomata both surfaces	Average stomata per square millimeter of leaf surface based on average for both surfaces	Rust reaction	
	Width	Length	Apex	Middle	Base	Average			Prevalence	Type no.
Immune:	*Centimeters*	*Centimeters*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*	
Minnesota Selection C. I. 651	0.76	1.58					13	26	0	
Upper			14	14	12	13				
Lower			13	13	12	13				
Minnesota Selection C. I. 661	.75	1.56					16	32	0	0
Upper			17	16	16	16				
Lower			17	16	16	16				
Indian Type 46	.66	1.41					18	36	0	0
Upper			18	18	18	18				
Lower			17	17	18	17				
Resistant:										
Indian Type 68	.91	1.77					20	40	5	1
Upper			21	20	18	20				
Lower			20	21	18	20				
Bolley Golden C. I. 644	.78	1.42					19	38	50	1
Upper			19	20	17	19				
Lower			20	20	17	19				
Redwing C. I. 320	.75	1.36					19	38	15	1
Upper			20	20	18	19				
Lower			19	19	18	19				
Susceptible:										
Bison C. I. 389	.96	1.67					20	40	1–15	3–4
Upper			20	20	18	19				
Lower			20	20	19	20				
Pinnacle C. I. 693	.66	1.23					22	44	60	4
Upper			24	22	20	22				
Lower			24	23	20	22				
Crepitans C. I. 506	.70	1.59					22	44	70	4
Upper			24	23	20	22				
Lower			23	24	17	21				

MATURE LEAVES

Reaction, variety, and surface	Width	Length	Apex	Middle	Base	Average	Average both surfaces	Average stomata per sq mm	Prevalence	Type no.
Immune:										
Minnesota Selection C. I. 651	0.33	1.51					24	48		
Upper			25	25	25	25				
Lower			25	23	22	23				
Minnesota Selection C. I. 661	.42	1.67					24	48		
Upper			24	24	24	24				
Lower			25	23	22	23				
Indian Type 46	.34	1.61					23	46		
Upper			26	24	22	24				
Lower			23	22	21	22				
Resistant:										
Bolley Golden C. I. 644	.44	1.84					.28	56		
Upper			30	28	28	29				
Lower			29	26	26	27				
Redwing C. I. 320	.41	1.67					26	52		
Upper			28	26	26	27				
Lower			28	26	25	26				
Bison C. I. 389	.48	1.92					25	50		
Upper			26	25	25	25				
Lower			24	25	23	24				
Susceptible:										
Pinnacle C. I. 693	.39	1.48					28	56		
Upper			29	27	25	27				
Lower			32	26	25	28				
Deep Pink C. I. 648	.40	1.66					28	56		
Upper			30	29	28	29				
Lower			26	27	27	27				

Hursh (*22*), from a study of the nature of resistance of wheat to *Puccinia graminis tritici*, concluded that there was no relation between the stomatal number in wheats and their degree of resistance to stem rust. In the present experiments, although differences were observed in the number of stomata on leaves of different varieties of flax, they do not appear to be sufficiently significant alone to determine the resistance of a specific variety to rust. However, it should be pointed out that the differences in stomatal number of the varieties recorded are based on the number of stomata per square millimeter of leaf surface. If the total leaf area of plants of different flax varieties were considered, the differences in the total number of stomata on a plant such as the variety Deep Pink, and the total number on a plant of the variety Indian Type 46, might be quite significant. Although the presence of a greater number of stomata on the leaves of a certain variety might not necessarily render it more susceptible to rust than a variety with fewer stomata per leaf, such differences may possibly be of significance when correlated with other factors, such as stomatal movement. Furthermore, the presence of a greater number of stomata on the leaves of a susceptible variety may not be of importance when conditions for rust infection are at their optimum, but under conditions of low light intensity, low humidity, and scarcity of urediospores, it may be of considerable importance in determining the degree of infection on these leaves.

STOMATAL MOVEMENTS IN RELATION TO RESISTANCE

Several methods of observing changes in stomatal apertures were carefully considered, but owing to the small size of the stomata of the flax leaf most of these methods proved impractical. Thus, the cobalt-paper method of Trelease and Livingston (*43*), the various porometer methods of Darwin and Pertz (*9*), Knight (*24, 25*), and Laidlaw and Knight (*27*) are entirely unsuitable. The method of direct microscopical observation of the stomatal apertures of plants growing in the field, devised by Lloyd (*32*), is of limited practicability with flax, the small size of the stomatal aperture making it very difficult to distinguish between fully open and partially open stomata. The method of stripping off the epidermis from the leaf and quickly plunging it into absolute alcohol, also devised by Lloyd (*31*), was found to be the most practical for this investigation. The effect of absolute alcohol on strips of leaf epidermis is to dehydrate the cell wall and cause it to become stiff and hard before removing the water from the cell. As a result, the cell walls retain their original shape and the size of the stomata remains unaltered as long as the material is kept dehydrated. The reliability of this method has been verified by Loftfield (*33*), who compared the dimensions of the stomata of leaves in position with those of the stripped epidermis treated with absolute alcohol and found that the measurements were identical. On the strength of this evidence the method was adopted for the present work.

Strips of leaf epidermis were removed from several varieties of flax growing in the field, plunged immediately into absolute alcohol, and then stored in small vials to be examined microscopically in the laboratory later. Epidermal strips were removed from several ma-

ture leaves from different plants every hour during the course of the experiments. Temperature and relative humidity records were kept, together with observations on the hour of sunrise, intensity of light, and the duration of dew. The epidermal strips were examined microscopically in the laboratory, and camera lucida drawings were made of several typical stomata in each series. A typical stoma was then selected from this collection of tracings and transposed to a chart designed to depict diagrammatically and to compare the condition of the stomatal apertures of different flax varieties at different
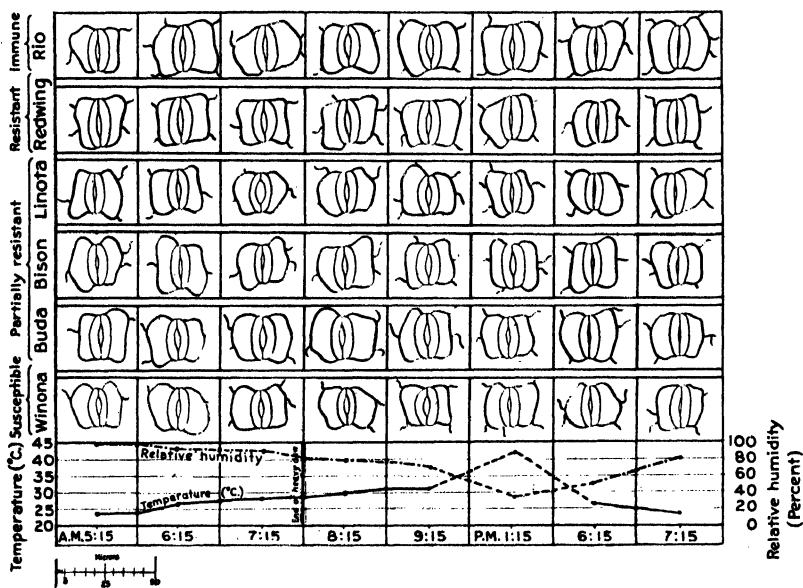
FIGURE 10.—Diagrammatic representation of the stomatal movement of different flax varieties under normal field conditions of light, temperature, and humidity at a time when conditions for infection were optimum. (Camera lucida tracings.)

periods of time. Observations were made from June 30, when conditions for natural infection by urediospores of rust were considered optimum.

As a preliminary experiment epidermal strips were removed from mature leaves of Winona, Buda, Bison, Redwing, and Rio flax plants in the preflowering stage, growing in the experimental plots at University Farm. The epidermal strips were handled in the manner outlined above, and a summary of the tracings obtained from these preparations is given in figure 10. It will be noticed that the stomata of the susceptible variety Winona were fully open at sunrise and remained so until noon, when they began to close, being reduced to narrow slits by late evening. In the partially resistant varieties, Buda, and Linota, the stomata were just beginning to open at sunrise, and attained their greatest aperture at about 7:15 a. m.; then they began to close, finally becoming reduced to narrow slits by 7:15 p. m. In the case of Bison, which is partially resistant to rust, the stomata opened rather slowly, and in this experiment did not become fully open until after the dew had evaporated, namely, at 9:15 a. m.

In the resistant variety Redwing the sequence was similar to that in the three previously mentioned varieties, the stomata opening rather slowly, becoming wide open at about 7:15 a. m., and commencing to close at 1:15 p. m. In the immune variety Rio, the stomata opened comparatively slowly, attaining their greatest aperture at 9:15 a. m., after the dew had disappeared.

From this experiment it would seem that at no time while the dew was on the leaves and while conditions were at their optimum for infection were the stomata of any variety closed sufficiently to prevent the entrance of the urediospore germ tubes. The stomata of all varieties, with the possible exception of Bison, had opened to approximately half their maximum aperture by the hour of sunrise, and this would not seem to interpose an effective barrier to germ-tube penetration.

At this point another experiment was made to determine whether there was any difference in the stomatal movement of the upper and lower leaf surfaces of the flax plants. Using the same technic as in the previous experiment, the strips of leaf epidermis were removed at hourly periods from the upper and lower leaf surfaces of Bison and Rio flax plants growing in the experimental field plots. Camera lucida tracings of the stomata of these samples were made and representative specimens are shown in figure 11, *A*. From a study of this chart, it is apparent that there is relatively little difference between the stomatal movements of the upper and lower leaf surfaces. The stomata of the lower leaf surface do appear to lag behind those of the upper surface slightly and perhaps do not open as wide, doubtless because the sun's rays hit the upper leaf surface first, and the amount of light received by the upper surface must be more than that which reaches the lower leaf surface, because of the manner of attachment of the leaf to the stem.

In a previous experiment the stomatal movements of several flax varieties were studied under conditions of bright sunlight and heavy dew. It was thought desirable to study the stomatal movements under the other extreme of conditions. Accordingly, the stomatal movements of the upper epidermis of Winona, Bison, Pale Blue, and Redwing flax were studied on a cloudy day when there was no visible dew and the light intensity was low. The movements of the stomata on the upper epidermis of the four varieties mentioned are illustrated in Figure 11, *B*. Again it will be noticed that the stomata of all of the varieties were approximately half open at sunrise, and a comparison with figure 10 would indicate that low light intensity and low humidity did not materially influence stomatal movement in this instance.

EFFECT OF DARKNESS DURING THE INCUBATION PERIOD ON RUST DEVELOPMENT

Although there are indications that stomatal movement in certain varieties may operate in resistance, it is not possible, on the basis of the foregoing experiments, to state definitely what effect stomatal movements may have in determining the resistance of certain varieties to rust. Since the methods used in these experiments were rather crude and decidedly unnatural it was considered advisable to approach the problem in a different manner.

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Accordingly, seeds of Winona, Redwing, and Buda were planted in the greenhouse, and when the seedlings had attained a height of approximately 8 inches, one series was placed in an incubation cham-
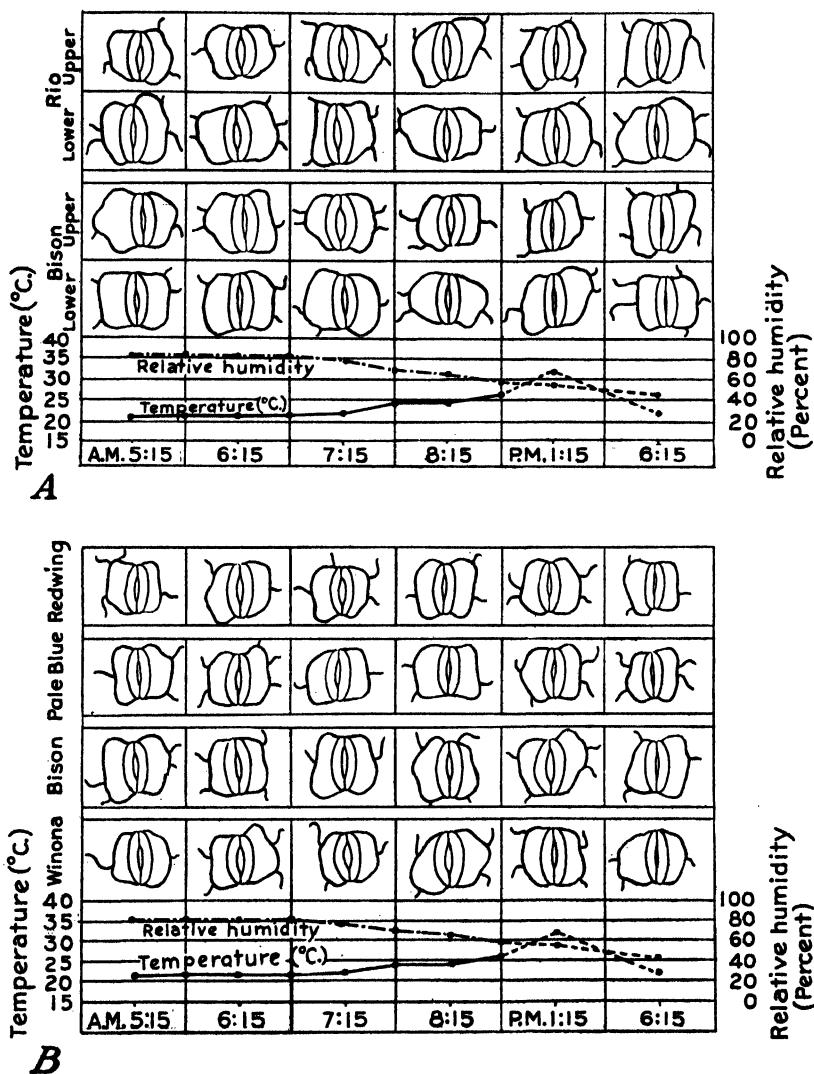
FIGURE 11.—Diagrammatic representation of the stomatal movements of different flax varieties under field conditions. (Camera lucida tracings.) *A*, Comparison of the stomatal movement of the upper and lower leaf surfaces of Bison and Rio flax; *B*, stomatal movements of the upper leaf surface of Winona, Bison, Pale Blue, and Redwing flax under conditions of low humidity and low light intensity.

ber from which all light was excluded by means of sheets of heavy paper, and a second series of the same varieties in the same stage of maturity was placed in another chamber from which the light was not excluded. The plants were left in these chambers for 24 hours and were then uniformly inoculated. The inoculated plants

were then incubated for an additional 48 hours. Both series were
kept side by side in the greenhouse during this period. The object
of this procedure was to determine whether closing of the stomata
by the exclusion of light would influence the development of rust.
Thus the plants in each series were incubated for 24 hours prior to
inoculation and 48 hours subsequent to inoculation, one series being
in total darkness during the entire 72 hours, the other being in nor-
mal diffused light for the same period. Forty-eight hours after
inoculation, the plants were removed from the incubation chambers
and placed side by side in the greenhouse. The plants were care-
fully examined for differences in vigor, but no differences were ob-
served. No visible symptoms of etiolation were apparent on plants
of the series incubated in the dark. Ten days after inoculation the
total number of leaves on each plant and the number of infected
leaves on the same plant were counted for each variety in both series.
The results given in table 7 are therefore the average of approxi-
mately 25 plants in every case. The number of uredia on individual
leaves of different plants also were counted; the figures for the aver-
age number of uredia per leaf given in table 7 represent the average
of approximately 25 leaves in each case. In determining the per-
centage of leaves infected, only green leaves susceptible to infection
at the time of inoculation were considered, the rosette of very young
leaves at the apex of each plant being excluded when the counts
were made.

TABLE 7.—*Effect of darkness during the incubation period on rust development
on three flax varieties*

| Variety | Rust reaction | Incubated in dark | | | Incubated in light | | |
		Total leaves per plant	Leaves infected per plant	Average pustules per leaf	Total leaves per plant	Leaves infected per plant	Average pustules per leaf
		Number	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*	*Number*
Winona	Susceptible	16	11	69	5	19	16	84	9
Redwing	Partly resistant	38	12	32	2	59	47	80	7
Buda	Resistant	53	3	6	1	44	8	18	2

From the results presented in table 7 it is apparent that darkness
during the incubation period resulted in a significant reduction in
the percentage of rust-infected leaves per plant and also in the
number of uredia developed per leaf, the series incubated in the
dark having consistently less rust than that incubated in normal
light. Furthermore, darkness during the incubation period in-
fluenced rust development on normally resistant varieties to a much
greater extent than it did on the susceptible variety Winona. Hart
(*16*) found that darkness does not influence the germination of
urediospores of *Melampsora lini* to any extent, and observations
by the writer corroborate her findings. The temperature in the
dark incubation chamber was about 3° lower than in the chamber
from which the light was not excluded (viz. 21° C.). This tem-
perature difference does not seem great enough to explain the fore-

going results, especially the difference in number of uredia. The
humidity in the two chambers appeared to be approximately the
same, or at least was adequate for optimum infection, and no dif-
ferences in the succulence of the plants in the two series were ap-
parent after incubation. Germination studies showed that the germ
tubes of the rust organism were not heliotropic as they ramified
in all directions irrespective of the source of light. Directing the
source of light so as to form a beam failed to result in a response
in the direction of germ-tube growth. Particular pains were also
taken to ensure uniform inoculation of all the plants in both series.

The elimination of the foregoing factors seems to indicate rather
strongly that the closing of the stomata of plants incubated in dark-
ness following inoculation with rust may possibly explain why
such plants were not so heavily infected as when they were incu-
bated in normal diffused light. These experiments were repeated
with similar results. It would be desirable, however, to check care-
fully the above findings, using additional varieties and plants in
various stages of maturity. In addition, attempts should be made
by means of microscopical observation of inoculated leaves in sur-
face view, to determine the actual percentage of germ tubes entering
the plants, and the percentage of germ-tube entrance through the
stomata should be compared on plants incubated in darkness and in
normal diffused light.

EFFECT OF FLAX WILT ON STOMATAL MOVEMENT IN RELATION TO RUST DEVELOPMENT

For a number of years certain experimental plots at University
Farm have been used for a study of flax wilt, caused by *Fusarium lini*
Bolley, and as a result the soil has become thoroughly infested with
inoculum. It is rather a striking fact that flax rust has not, up to the
present time, been noticed on the flax varieties growing in these wilt-
infested plots. The nonoccurrence of flax rust in these plots may
have been due to a number of causes. (1) The humidity in this
locality at the time when infection is likely to occur may have been
too low to permit successful infection. This seems unlikely, as leaf
and stem rust of wheat have been very prevalent in the immediate
vicinity. (2) It may have been due to an absence of sufficient inocu-
lum, which does not seem probable, as epidemics of flax rust have
occurred during the past 3 years, at least on plants growing within
a distance of 1 mile, and one would expect that sufficient urediospores
would be blown from these infected plants to cause the appearance of
at least some rust in the flax-wilt plots. (3) The failure of rust to
appear in these plots may have been due to certain soil factors which
were unfavorable for the development of rust, and which might also
have resulted in the failure of the stomata to open, thus preventing
the entrance of urediospore germ tubes of flax rust. The plants in
these plots are planted in short, widely separated rows, which would
probably reduce the rate of spread of the disease but certainly would
not prevent its appearance.

On June 27 the stomatal movements of a number of flax varieties
in the early-blossom stage in wilt-sick soil were examined at hourly
intervals, using the technic previously described. Observations were

made on the following varieties: Buda, Bison, Pale Blue, Winona, Redwing, Linota, and Pale Pink. The plants, although growing in wilt-sick soil, at this time had no symptoms of wilt and appeared to be healthy and turgid. When epidermal strips were removed from the plants and examined under the microscope, it was found that, in most varieties, stomata were completely closed at all times, while in the remaining varieties they apparently had never opened to more than a narrow slit. At first it was thought that the technic used in removing and fixing the epidermal strips might have been faulty, causing the collapse of the guard cells and the subsequent closing of the stomata. The experiment was therefore repeated, dehydrating the epidermal strips in fresh absolute alcohol, but the results were the same. These observations were repeated several times and checked with observations of the stomata of flax plants growing in healthy soil. In all cases the stomata of the plants growing in the wilt-sick soil apparently failed to open, whereas those of plants growing on healthy soil and examined at the same time opened in the normal way.

It would thus appear possible that the presence of *Fusarium lini* in the soil resulted in the death of the guard cells and the subsequent nonfunctioning of the stomata of the plants concerned.

Iljin (*23*), in 1922, reported that in certain plants which were suffering from physiological wilt, the guard cells invariably died and the stomata ceased to function.

In the light of the above observations it would seem possible that the failure of flax rust to appear in the wilt-sick plots was due to the fact that the stomata of the plants growing in this soil for some reason failed to open and thus prevented the germ tubes of flax rust from entering. The nonfunctioning of the stomata of these plants may in turn have been due to the effect of flax wilt on the physiology of the host plant.

TO WHAT EXTENT IS FLAX RUST RESISTANCE INFLUENCED BY NUMBER AND MOVEMENTS OF STOMATA?

In the preceding investigation of the stomata of the flax plant indications were obtained that certain immune and resistant varieties have fewer stomata per square millimeter of leaf surface than certain other varieties susceptible to rust. If one takes into consideration the total leaf surface of such plants it is possible that the differences in number of stomata on resistant and susceptible varieties may be of significance in germ-tube penetration in the former varieties, inasmuch as the germ tubes of urediospores enter the plant through the stomata.

Observations of stomatal movements of a number of flax varieties did not uncover striking differences in the stomatal movements of varieties differing in their reaction to rust. However, there were indications of slight differences in the rate of opening of the stomata of certain varieties. Such differences might well be a factor to be considered in view of the fact that the time during which germ-tube penetration can take place is of relatively short duration, being confined to the period when the dew is still on the leaves and while the stomata are sufficiently open to allow the entrance of the uredio-spore germ tubes, a period of approximately 3 to 4 hours, except,

of course, in case of rains. In a susceptible variety such as Winona the stomata have already attained their maximum width by the time the sun rises, and the opportunities for germ-tube entrance are favorable from the hour of sunrise, or from the time of stomatal opening, until the dew disappears from the leaves. In a variety such as Linota, on the other hand, the stomata do not attain their maximum aperture until 1 to 2 hours after sunrise and then remain fully open for 1 hour only. Thus the opportunity for germ-tube entrance by way of the stomata is confined to the brief period of 1 hour. It would seem that the chances of the germ tubes being able to grow sufficiently to enter a stoma during this period are comparatively slight. In the variety Bison the stomata do not open to their maximum width until the dew has disappeared from the leaves, and at this time conditions for germ-tube growth are decidedly unfavorable because of an inadequate film of moisture on the leaves. In the field Bison has been found to be partially resistant to rust, having the reaction 15 percent type 3 to 4, which means that approximately 15 percent of the total leaf surface was occupied by type 1 uredia. It is reasonable to suppose that the failure of the stomata of Buda to open until after the dew had evaporated may explain the low percentage of rust which normally develops on this variety in the field. It has not been determined whether urediospore germ tubes of *Melampsora lini* can penetrate closed or partially closed stomata, but it does seem entirely reasonable to suggest that partial closing of the stomata would offer at least some barrier to entrance of germ tubes.

When inoculated plants were incubated in darkness, there was decidedly less rust than on plants incubated in the light. By a process of elimination of several possible explanations for the above phenomenon it appears that the closing of the stomata on plants incubated in the dark may have been responsible. Here, apparently, is another indication that stomata of the flax plant may operate in influencing rust development. An examination of stomata of flax plants growing in wilt-infested soil revealed the fact that they were apparently closed at all times during the period of observation throughout the day. For a number of years rust has failed to develop in these plots, and the suggestion is advanced that the atrophy of the stomatal guard cells and the consequent closing of the stomata, possibly due to the presence of *Fusarium lini* in the soil, may have been responsible for the failure of rust to appear in these plots.

From a careful consideration of the above facts, one is forced to conclude that the stomata of the flax plant may, under certain conditions and with certain varieties, play a role in rust resistance. However, it should be pointed out again that differences in stomatal movements do not alone account for the susceptibility or resistance of varieties to rust.

It seems extremely important, when considering the role of stomata in plant disease resistance, to bear in mind the probability that under conditions optimum for infection, that is, when adequate moisture is present, when there is an abundance of inoculum, and when light conditions are favorable for normal stomatal movement, stomata may not be of much importance as determiners of the

resistance or susceptibility of a specific variety; however, when optimum conditions for infection do not prevail, that is, when dew and rain are absent, when urediospores are not abundant, and when low light intensities prevail, then stomata may be of considerable importance in influencing the amount of infection that is likely to occur on certain varieties.

EFFECT OF NUTRIENT SALTS ON RUST DEVELOPMENT

It is a commonly observed fact that the succulence and vigor of the host plant profoundly affect the susceptibility of the plant to rust, the more succulent and vigorous plants in general being more susceptible to rust. Hursh (*22*), working on the nature of resistance of wheats to stem rust (*Puccinia graminis tritici*), found that plants heavily fertilized with nitrogen may be more severely injured by stem rust than those not so fertilized. Heavy nitrogen fertilization was found to decrease the amount of sclerenchyma in proportion to the amount of parenchyma present in the wheat stem. Hart (*17*), contrary to the results obtained by Hursh, concluded from her experiments that there were no consistent or significant differences in the proportion or distribution of collenchyma in the peduncles of wheats grown in different fertilizer plots.

In this work attempt was made to determine whether the application of mineral salts would affect the development of rust on normally resistant flax varieties. Seeds of Winona, Redwing, Buda, and Rio flax were planted in 6-inch plots in sand, and until the time of emergence of the seedlings, were watered in the usual manner. Subsequent to the time of emergence, the plants were watered with the following nutrient solutions containing the indicated quantities of salts per 1,000 cc. of distilled water:

a. Sach's solution: *Grams*
    Potassium nitrate_____ 0.4
    Calcium sulphate_____ .5
    Calcium phosphate_____ .5
    Sodium chloride_____ .5
    Magnesium sulphate_____ .5
    Calcium carbonate_____ .5
b. Sach's solution plus excess nitrate:
    Potassium nitrate_____ 1.2
    Calcium sulphate_____ .5
    Calcium phosphate_____ .5
    Sodium chloride_____ .5
    Magnesium sulphate_____ .5
    Calcium carbonate_____ .5

c. Sach's solution plus excess phosphate: *Grams*
    Potassium nitrate_____ 0.4
    Calcium sulphate_____ .5
    Calcium phosphate_____ 1.5
    Sodium chloride _____ .5
    Magnesium sulphate_____ .5
    Calcium carbonate_____ .5
d. Sach's solution plus excess potassium:
    Potassium nitrate_____ .4
    Calcium sulphate_____ .5
    Calcium phosphate_____ .5
    Sodium chloride_____ .5
    Magnesium sulphate_____ .5
    Calcium carbonate_____ .5
    Potassium sulphate_____ .5

Duplicate pots of each variety were used for the four series, and the nutrient solutions were applied by means of a pipette at the rate of 50 cc. per pot per day. Precautions were taken to ensure uniform stands of each variety in all pots.

Three weeks after planting, notes were taken on the height and succulence of the plants of each variety watered with the different nutrient solutions. The plants were then inoculated with fresh urediospore material, care being taken to inoculate each pot uniformly,

and they were then incubated in the usual way for 48 hours. Ten days after inoculation, notes were taken on the relative development of rust on the different varieties in each series. Counts were made of the total number of leaves per plant, the number of infected leaves per plant, and the number of uredia per leaf. The results, given in table 8, are thus the average for both pots of each variety, and the average number of uredia per leaf are the averages of approximately 25 leaves in each case.

TABLE 8.—*Effect of mineral salts on rust development in three flax varieties*

Variety	Nutrient solution	Average height of plants	Total leaves per plant	Infected leaves per plant		Average uredia per leaf
		Centi-meters	*Number*	*Number*	*Percent*	*Number*
Winona	a. Sach's solution	8.8	40	32	80	16
	b. a+excess nitrate	11.6	40	34	85	14
	c. a+excess phosphate	10.6	40	34	85	20
	d. a+excess potassium	9.2	40	32	80	9
Redwing	a. Sach's solution	9.2	36	20	56	7
	b. a+excess nitrate	9.0	37	24	65	10
	c. a+excess phosphate	10.5	39	32	82	14
	d. a+excess potassium	8.2	34	14	41	4
Buda	a. Sach's solution	9.3	43	4	9	2
	b. a+excess nitrate	11.5	41	13	32	10
	c. a+excess phosphate	12.5	44	22	50	16
	d. a+excess potassium	9.5	42	20	48	7

The different nutrient solutions were found to influence the succulence and height of the plants to a noticeable degree. Excess phosphate especially seems to favor the growth of flax, the plants in the phosphate series being much more succulent and more vigorous than those watered with ordinary Sach's solution, and with Sach's solution plus excess potassium. The plants watered with the last two nutrient solutions were poorly developed and far less succulent than those watered with the nutrient solution containing excess phosphate and the solution containing excess nitrate. Excess potassium appeared to stimulate the growth of the plants very slightly, with the exception of Redwing, but the plants in this series were also poorly developed and less succulent than plants in the excess phosphate series. Excess nitrogen stimulated growth, and, in addition, the plants so treated were very vigorous and quite succulent. There appeared to be a differential response of certain of the varieties to the different nutrients, excess nitrate being most favorable for the growth of Winona, whereas excess phosphate was most favorable for the growth of Redwing and Buda. The effects of the different nutrient solutions on rust development are shown diagrammatically in figure 12.

It will be noticed from figure 12 that the application of excess phosphate favored rust development in all varieties. Excess nitrogen also stimulated rust development over the check series (ordinary Sach's solution), and excess potassium reduced the amount of rust development in Winona and Redwing, but not in Buda. In the case of Buda, excess potassium stimulated the growth of the plants slightly, which may account for more rust development on the Buda plants treated with excess potassium than on plants of the same variety treated with ordinary Sach's solution.

The interesting point in these results is the fact that the various nutrient solutions did not significantly alter the rust development on the normally susceptible variety Winona, but the application of excess phosphate did appear to influence significantly the susceptibility of the normally resistant varieties Redwing and Buda. Phosphate and nitrate application also increased the number of uredia produced on these two varieties. Increased susceptibility to rust of plants watered with excess phosphate and with excess nitrate is correlated with increased growth and succulence of the plants concerned. The
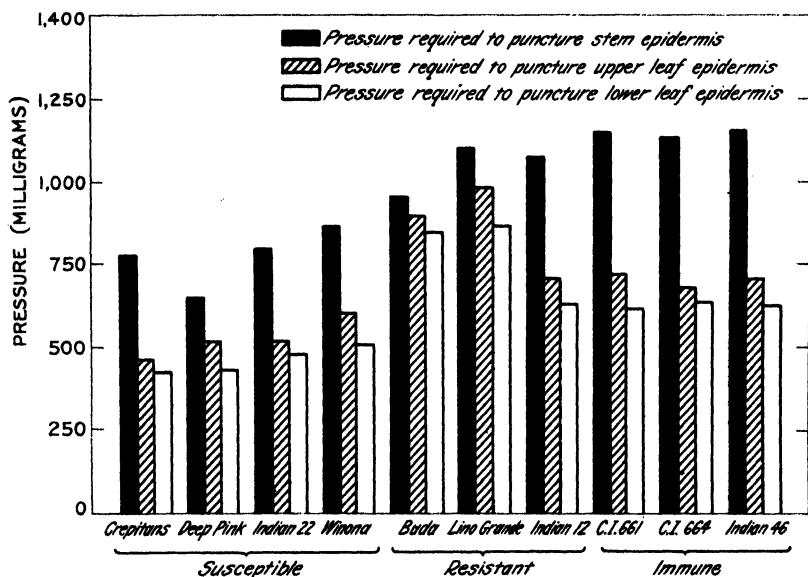
FIGURE 12.—Effect of nutrient salts on rust development in three varieties of flax.

different nutrient solutions failed to render susceptible the immune variety Rio.

From these results it appears that the application of excess nitrate and excess phosphate increased the development of rust, and excess potassium reduced it in the varieties studied.

DISCUSSION AND CONCLUSIONS

It is obvious that the resistance of cultivated flax to flax rust, *Melampsora lini*, is an extremely involved problem. The histological work demonstrated that certain varieties of flax exerted no protoplasmic antagonism toward the rust fungus, allowing the latter to develop extensively and to culminate its activities by the production of the overwintering teliospores, without encountering any apparent nutritional difficulties. Certain other varieties, on the other hand, appeared to erect nutritional barriers to the development of the rust fungus, restricting its development to a certain degree, but yet allowing it to complete its life cycle by the production of spores. Although the spores produced may be relatively few in number, they do nevertheless suffice to ensure the perpetuation of the species if suitable infection courts exist. Such varieties are

obviously resistant to rust, and the incompatability of host and parasite is evidenced by the necrosis of the host tissues.

In other varieties the protoplasmic antagonism of the host toward the fungus constitutes a barrier that the pathogen apparently cannot surmount, restricting its activities to the development of a sparse hyphal system and entirely preventing it from ensuring its perpetuation by the production of urediospores. The incompatibility of host and parasite in these varieties is evidenced by the complete necrosis of the host tissues in the infection zone, and by the failure of the rust to produce urediospores. Such varieties are considered to be highly resistant to flax rust.

In flax varieties immune to attacks by the rust fungus, the incompatibility between host and parasite is so complete that the latter fails to develop sufficiently to make its presence externally evident, even when it has gained entrance to the interior of the host.

Plant extracts obtained from varieties susceptible, resistant, and immune to rust were found to support the growth of urediospore germ tubes of the rust organism in precise agreement with the rust reactions of the varieties from which they were obtained. Thus it is obvious that the protoplasmic properties of the different flax varieties influence to a marked degree the ability of the rust fungus to establish itself and complete its normal cycle of development. In certain cases such properties may operate alone in determining the immunity of certain varieties; in other cases they apparently operate to a more limited extent and are not alone sufficient to prevent the development of rust. It must therefore be admitted that physiological properties of the host do operate in determining the resistance or susceptibility of a variety to rust, and further investigations of a physiological or serological nature would be desirable.

With regard to the role of morphological characters in resistance, it has been shown that there do not appear to be any gross anatomical features of the flax plant that can be considered as determiners of resistance. It has been demonstrated that the epidermis of varieties resistant to or immune from rust, without exception in the varieties studied, was far more resistant to puncture, as determined by a modified Joly balance, than was the epidermis of some susceptible varieties. Such a resistance to puncture was found to be correlated with the presence of a cuticle, isodiametric epidermal cells, and the development of hypodermal layers. It has previously. been pointed out that such an epidermis, resistant to puncture, may operate in one of two ways, or in both, in influencing the resistance of a specific variety to rust. It has been suggested that an epidermal membrane resistant to puncture may prevent the entrance of sporidial germ tubes of the fungus just as that of certain immune varieties of barberry prevents entrance of those of *Puccinia graminis tritici*. Also, it has been shown that certain varieties possessing an epidermal membrane capable of withstanding relatively high tensile strains quite commonly develop subepidermal uredia and fail to liberate many of their urediospores. It is suggested that this fact would significantly reduce the amount of available inoculum pro-

duced in the course of the summer, provided these varieties were grown to any extent, and it may therefore be of considerable practical importance.

Thus it has been shown that morphological features of the host plant in certain cases play a part in the resistance of certain varieties to rust. While such factors do not seem to operate alone, it must be conceded that they contribute their share to the resistance of some flax varieties to rust.

Although no wide differences in the stomatal movements of different flax varieties were observed, indications were obtained that the rate of opening of the stomata may explain the low percentage of rust development on certain varieties. The fact that inoculated plants incubated in the dark developed less rust than similar plants incubated in the light would seem to support the opinion that the stomata of flax may play a part in resistance to rust. The fact that varieties of flax growing in soil infested with *Fusarium lini* have failed to rust for a number of years is interesting and significant in view of the observation that inoculum is abundant in this locality and conditions for infection are normally favorable. The observations of the stomata of these plants revealed the remarkable fact that they were apparently functionless because of certain soil factors or because of the presence of *F. lini* in the soil. It is suggested that this apparent nonfunctioning of the stomata may possibly explain the failure of rust to appear on plants growing in these plots. Again, this would seem to be additional evidence for the role of stomata as "cooperators" in influencing susceptibility to rust. Thus it must again be conceded that there is evidence that the stomata of the flax plant may contribute towards rust resistance.

In this investigation it was repeatedly observed that certain varieties resistant to rust in the field may be susceptible under greenhouse conditions. It has been shown that the application of certain nutrient salts, e. g., nitrate and phosphate, will favor the development of rust on varieties normally resistant under field conditions. In addition, plants in early stages of maturity appear to be more susceptible to rust than fairly mature individuals. This would suggest that environmental conditions and the general state of vigor of the plants undoubtedly influence the reactions to rust, and an investigation of a physiological nature along these lines would be desirable.

It has been shown that morphology, physiology, and stomatal movements of the flax plant may, under certain conditions and in certain varieties, all contribute their share to the resistance of a specific variety to rust. Certain of these factors may be of minor importance when optimum conditions for the development of rust prevail, but one should give very careful consideration to the possibility that they may be of great importance when optimum conditions for rust development do not prevail.

The resistance of cultivated flax to *Melampsora lini* cannot be attributed to any one single factor operating alone but must be considered to be due to a number of factors operating together to produce by their united efforts the condition termed "flax rust resistance."

SUMMARY

Flax rust, caused by *Melampsora lini* (Pers.) Lév., may be considered to occur wherever cultivated flax is grown.

The disease annually causes a reduction of approximately 2 percent of the total yield of seed flax in the United States.

Varieties of flax may react in five different ways to rust in the field, such varieties being distinguished by the type of uredium produced. Flax may be immune, highly resistant, resistant, incompletely susceptible, or completely susceptible to rust, the various classes being designated by the numerals 0, 1, 2, 3, and 4, respectively.

The development of flax rust within the tissues of a susceptible variety was studied and illustrated in detail.

Histological studies of uredial types characteristic of the five classes of rust reactions indicate that the physiology of the host may influence the resistance or susceptibility of the variety to rust.

Plant extracts obtained from varieties of flax differing in their reaction to rust supported the vegetative growth of urediospores of *Melampsora lini* in accordance with the resistance or susceptibility of the varieties from which they were obtained.

There are no gross anatomical features of the flax plant which may be considered to operate alone in the determination of flax rust resistance.

The thickness of the epidermal membrane of leaves and stems of different flax varieties may be of considerable importance in uredium formation and urediospore liberation. Determinations by means of a modified Joly balance of the pressures required to puncture the stem and leaf epidermis showed that varieties resistant or immune to rust possessed an epidermis which required significantly more pressure to effect puncture than did the epidermis of certain susceptible varieties.

The resistance of the epidermal membrane to puncture is correlated with the possession of a cuticle, the development of a hypodermis, and the isodiametric shape of the individual epidermal cells. The epidermis of susceptible varieties, in general, lacks a well-developed cuticle, the individual epidermal cells are rectangular rather than isodiametric, and the hypodermis is usually absent. The strength of the epidermal membrane as indicated by resistance to puncture may determine the ability of the rust fungus to develop uredia and to liberate its urediospores in certain varieties. Such a condition may reduce the amount of available inoculum produced in the course of the summer.

The size, shape, and arrangement of the cortical fibers cannot be correlated in any way with rust resistance.

Certain flax varieties susceptible to rust possess a larger number of stomata per square millimeter than certain other varieties resistant to or immune from rust. This may be of significance in influencing the percentage of germ-tube penetration.

Stomatal movements may operate in rust resistance. The stomata of the variety Bison, resistant to rust in the field, did not open until after the dew had disappeared from the leaves. This may account for the low percentage of rust development on this variety in the field.

The presence of *Fusarium lini* in the soil may account for the failure of flax rust to appear on the varieties growing in wilt-sick soil, possibly because of the atrophy of the guard cells and the failure of the stomata of these varieties to function.

Darkness during the incubation period apparently suppressed the development of rust. This fact may furnish additional evidence in support of the theory that stomatal movements play a role in the resistance of certain flax varieties to rust.

The application of different nutrient salts influenced rust development. The application of excess nitrogen and excess phosphate increased rust development, while excess potassium appeared to suppress it somewhat. The reaction of a normally susceptible variety was not significantly altered by the application of different nutrient salts, but the development of rust on the normally resistant varieties Buda and Redwing was greatly increased when excess nitrate and excess phosphate were applied.

The resistance of cultivated flax to flax rust cannot be attributed to any one single factor operating alone but must be considered as due to a number of factors operating in conjunction with each other.

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RESPONSE OF WHEAT VARIETIES TO DIFFERENT FERTILITY LEVELS[1]

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INTRODUCTION

The adaptability of a variety of any of our common crops is definitely limited. Climate is probably the most important single factor in setting the boundaries within which a variety may be successfully grown. To weather conditions also are attributable in large measure the great variations in yield between one season and another. Within a given region, type and condition of soil become important, and in some cases are deciding factors in the choice of the strain to be grown.

The question arises: Is it reasonable to expect a winter wheat, developed and tested under conditions of rather high fertility, to maintain its relative standing when grown on soils of medium or low fertility, even of the same general type? Some selections may be better adapted to rich and others to poor soils. Obviously, this has a direct bearing on wheat-breeding and testing programs.

The present study is concerned with the response of a number of wheat varieties and strains to different fertility levels. Since the experiment extends over a 5-year period, a considerable amount of the variation in yield is due to season. For this reason, a rather critical review of the climatological data is necessary. Since, however, it is merely sought to eliminate, as far as possible, the responses to climate and study the responses to fertility levels alone, a literature review on this topic is not considered necessary. A large number of citations may be found in Hannay's bibliography of the influence of weather on crops (*3*).[3]

REVIEW OF LITERATURE

The study of differential reaction of varieties to fertility level has received little attention. Fertilizer tests are generally confined to one or two varieties, and variety tests are usually conducted at only one fertility level. A few experiments, however, do bear directly upon the problem in hand. Weigert and Fürst (*9*) make the point that farmers should choose varieties on the basis of their reaction under the conditions existing on their particular farms. This recommendation is based upon results of tests with winter rye, winter wheat, spring barley, and oats which showed differential response to fertilization. Gregory and Crowther (*2*) ran carefully controlled pot experiments with five pure line varieties of two-rowed barleys varying not

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only in available nutrients but also including certain "deficiency" series where one essential element—nitrogen, phosphorus, or potassium—was supplied in less than optimum amount. They found a differential response to change of manuring, and conclude that varietal and manurial trials should be combined. They also note that the possibility exists of developing varieties suited to deficient soil types. Beaven, in an appendix to this article, confirms the general conclusions from results of his field trials.

Other less specific references are also to be found. Hunter and Leake (*4*), in discussing British wheats, point out that Yeoman will rank in yield with the best of the softer varieties only when grown on soil in a high state of fertility. They further state: "No hard wheat has been obtained yet that is equal to Squarehead's Master on soils in a medium condition of fertility" (*4, p. 23*). Referring to the Dutch variety Wilhelmina, they say: "* * * it is a prolific grain producer under what may be termed average soil conditions * * *" (*4, p. 31*). In discussing Swedish wheats, they point out that, among spring wheats from progeny of Kolben and Extra-Kolben × Schlanstedter (a variety popular in Saxony), lines were obtained which "* * * were characterized by a distinct tendency towards the requirement of special conditions to enable them to produce their maximum results * * *" (*4, p. 38*). The exact conditions referred to, however, are not stated.

Immer, Hayes, and Powers (*5, p. 418*) made a study of the adaptation of barley varieties, from which they concluded that—

some varieties reacted differently in different stations, in different years, and in certain stations in specific years but * * * some varieties were significantly superior to others in spite of the differential responses.

Mooers (*6, p. 800*), studied the reaction of a number of corn varieties to two levels of fertility and concluded that—

Varietal trials on soils of both high and low productivity are required in order to furnish a comprehensive picture of the adaptability and relative standing of different varieties.

Stringfield and Salter (*8*), reporting on the corn crop in the same rotation with which the present paper is concerned, found a differential response of variety to season and to fertility level.

Other citations might be made, but the foregoing indicate that the problem of varietal response to fertility level is one worthy of attention wherever the results of variety tests are to be applied to a wide range of conditions.

OUTLINE OF EXPERIMENT

A 3-year rotation of corn, oats, and winter wheat was started in 1928 at the Ohio Agricultural Experiment Station, Wooster, to study the response of a number of varieties of these crops to different fertility levels.[4] Wheat only is considered in this paper.

The plots were laid out in three blocks, separated by short distances, but all were located on Canfield silt loam. Conditions of slope and drainage varied somewhat. Since the Canfield silt loam was naturally infertile, it was possible to build up a series of fertility levels by adding fertilizers and manure. Level A, receiving no

plant nutrients in any form, was poorest. Levels B, C, and D provided increasingly productive conditions obtained by adding one, two, and four increments of fertilizing materials. The applications are given in table 1.

TABLE 1.—*Plot treatments at the 4 fertility levels used in 3-year rotation tests with corn, oats, and wheat*

Level	Treatment used for crop indicated		
	Corn	Oats	Wheat
A	None	None	None.
B	4 tons manure, 100 pounds 0-16-0 broadcast, 100 pounds 4-12-4 in hill.	do	200 pounds 2-14-4 in fall, 50 pounds nitrate of soda in spring.
C	8 tons manure, 200 pounds 0-16-0 broadcast, 200 pounds 4-12-4 in hill.	do	400 pounds 2-14-4 in fall, 100 pounds nitrate of soda in spring.
D	16 tons manure, 400 pounds 0-16-0 broadcast, 400 pounds 4-12-4 in hill.	do	800 pounds 2-14-4 in fall, 200 pounds nitrate of soda in spring.

The four levels were arranged on adjacent strips and the varieties sown across them. Wheat was planted in triplicate, the size of plot on each level being 5⅓ by 35 feet (0.00427 acre). Plots were harvested separately; the sheaves were weighed before and the grain after threshing. Weight per bushel was determined for each variety at each level.

PRESENTATION OF DATA

From 15 to 17 winter wheats were grown each year for harvest from 1929 to 1933, inclusive. Of the 20 varieties and strains used, 11 were grown all 5 seasons. Table 2 gives the grain yield, straw yield, and weight per bushel for these 11 lines. Through an error, the sheaves were not weighed before threshing in 1930, and grain yield alone was available for that year. Figure 1 presents graphically the averages for the 11 lines.
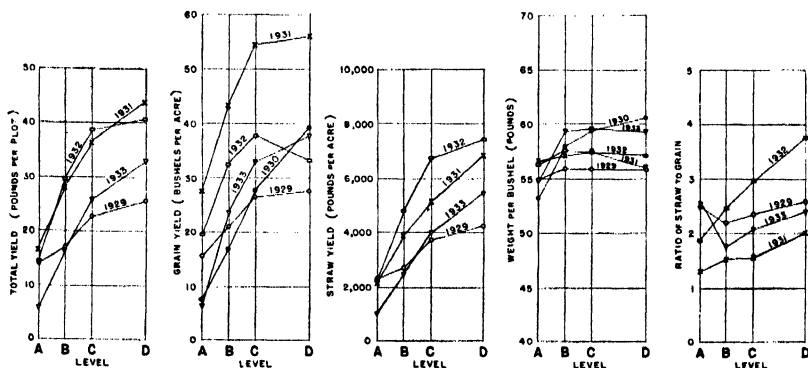
FIGURE 1.—Average response of 11 varieties of winter wheat to 4 soil-fertility levels in 5 seasons.

The plots were cut with an ordinary grain binder, so that uniform stubble was left on the field. This, of course, affected the straw

yields, making them relatively too low on level A, and, in cases where the straw was leaning somewhat, also on level D.

From a study of figure 1, it is clearly evident that season plays an important part in the behavior of winter wheats.
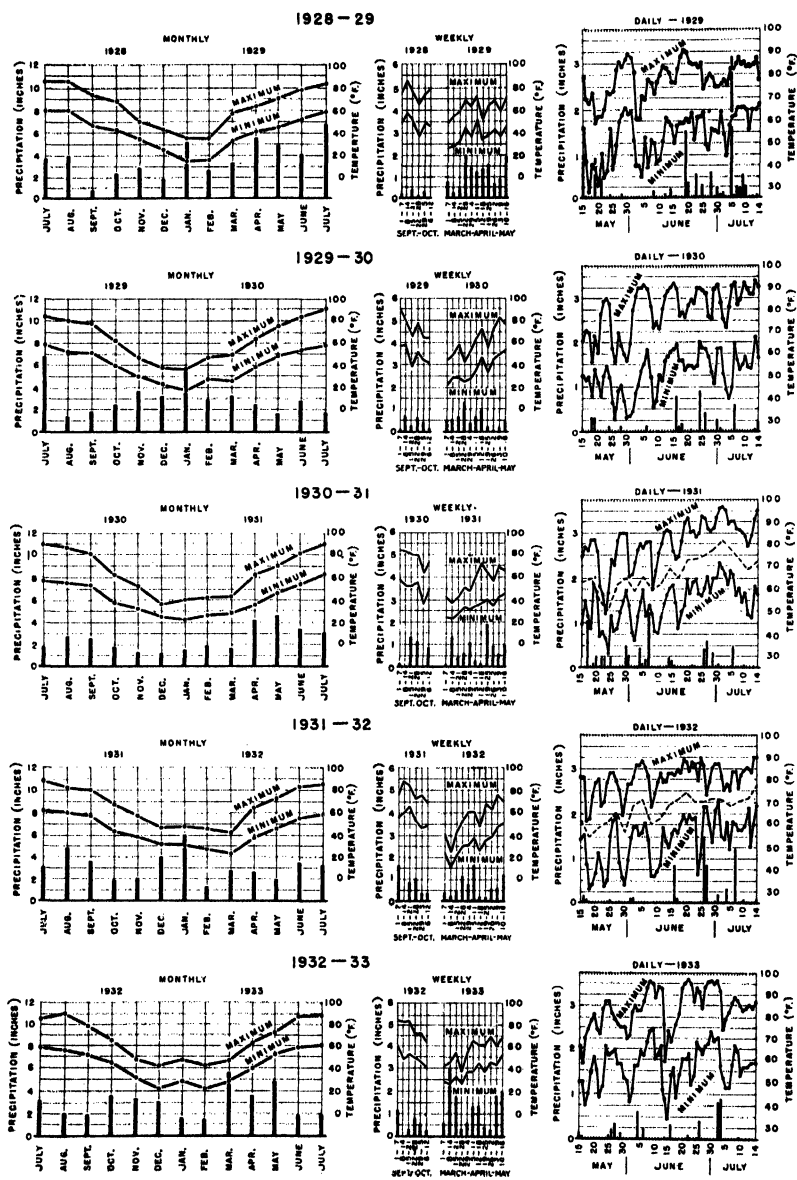
FIGURE 2.—Meteorological data for the period of the experiments, Wooster, Ohio, 1928–33.

Climatological data are presented in figure 2. The graphs show the mean maximum temperatures, mean minimum temperatures, and the precipitation by months for 13-month periods, July to

July, inclusive. In addition, these data are given in greater detail for periods of especial importance to the wheat plant; viz, weekly before and after seeding time and again during the spring growing period, and daily from heading to harvest.

DISCUSSION OF DATA

Variations in yield from one season to another are due largely to climatic factors. Other considerations, certainly of minor importance, include the differences between soil blocks, and on the higher fertility levels in the later years, the residual effects of fertilizers, especially the stable manure applied to the corn.

To obtain maximum yields there must be sufficient moisture and nutrients available to produce strong, healthy fall growth in order that the wheat plants may withstand the winter and be in condition to make a quick start in the spring. During the spring the plants must make the remainder of their vegetative growth and take from the soil the great bulk of the mineral elements which are utilized later in grain development.

At Wooster, factors of prime importance include moisture relations in the fall and early spring, temperature during these periods and also during the winter, and weather conditions at critical periods, such as heading time and the final desiccation period when the kernels are hardening.

Winter injury did not occur to any appreciable extent in any year under consideration. Lodging was not serious, even on level D with heavy applications of nitrate of soda. Hence, these factors need not be considered as influencing the results.

A brief review of weather conditions and crop responses in each of the five seasons as an aid to the interpretation of the statistical analysis of the data follows. The average of the 11 strains grown in all seasons (table 2 and fig. 1) is used as a basis for these observations.

THE 1928-29 CROP

In 1928 September was exceptionally dry, and in October there was only normal rainfall. Wheat did not make good fall growth, and, therefore, could not have taken up any large amount of nutrients before the onset of the dormant period. Spring rainfall in 1929 was very heavy, and probably resulted in some leaching. This was all the more serious since the plants had had an unfavorable start in the fall. Whether this accounted entirely for the lack of response to fertilizers in the 1929 crop is a question, but probably it was the most important consideration. The fact that wheat was the second crop of the rotation to be grown on this particular block and that there was no residual effect of manure and fertilizers used on the corn might have had some influence.

On level A, 1929 yields were low and the average increase from level A to level D was only 12.1 bushels of grain and 1,940 pounds of straw per acre. Straw to grain ratio and weight per bushel showed less variation across the levels than in any other season. This was not surprising in view of the small differences in yield.

TABLE 2.—*Yield per acre and weight per bushel of 11 varieties of winter wheat grown at 4 levels of soil fertility, 1928–29 to 1932–33, inclusive*

Variety [1]	Level	1928-29			1929-30			1930-31			1931-32			1932-33			5-year average		
		Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel [2]
		Bu.	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*
Michikof	A	19.2	2,420	59.2	2.7		40.0	24.5	2,640	57.8	14.8	1,800	59.2	3.4	700	54.0	12.9	1,800	54.0
	B	23.3	2,940	59.3	8.3		56.3	40.4	4,180	58.8	29.2	4,320	60.4	17.5	2,080	59.6	23.1	3,380	54.9
	C	26.0	2,700	59.1	16.0		57.4	40.4	4,920	59.0	34.2	5,980	60.6	23.2	3,100	60.4	29.7	3,400	59.4
	D	28.0	4,340	59.7	28.4		59.4	40.6	6,200	57.4	22.5	6,820	59.0	29.2	4,660	51.8	29.7	5,505	59.2
T. N. 1030 (Gladden × Trumbull)	A	18.0	2,580	55.0	10.1		55.0	30.1	2,000	54.0	17.4	1,840	55.3	2.8	600	58.1	15.7	1,755	54.2
	B	23.9	2,820	55.8	26.2		57.9	44.4	3,300	56.1	32.4	4,440	57.4	22.4	2,240	58.3	29.9	3,200	57.1
	C	31.5	3,720	56.2	38.6		60.4	44.4	4,680	57.1	39.9	6,400	58.4	36.3	4,080	—	39.7	4,720	57.9
	D	37.7	4,440	56.1	43.1		60.9	57.8	6,980	57.5	38.9	7,780	58.0	40.0	5,420	—	43.5	6,150	57.7
T. N. 1032 (Gladden × Trumbull)	A	17.4	2,300	57.5	9.6		57.8	23.5	2,080	58.9	19.7	2,320	58.2	7.3	1,080	56.6	15.5	1,945	57.1
	B	23.9	2,800	57.8	13.3		58.2	43.9	3,500	58.2	27.6	4,300	58.4	26.6	2,400	60.4	27.1	3,350	57.5
	C	30.5	3,880	57.7	26.9		60.2	52.8	4,840	58.7	37.9	6,120	58.7	35.0	4,080	60.2	36.6	4,730	58.7
	D	33.1	4,480	57.9	38.2		61.1	62.7	6,660	56.4	32.7	7,720	58.7	41.5	5,740	—	41.6	6,150	59.1
T. N. 1047 (Portage × Gladden)	A	16.8	2,320	55.7	9.7		54.0	31.5	2,320	57.1	20.5	2,500	57.1	5.5	1,000	54.3	16.8	2,035	58.9
	B	23.2	3,040	56.1	19.3		58.1	44.0	5,940	54.5	31.8	5,240	58.4	23.3	2,860	59.9	28.3	3,855	55.5
	C	22.9	3,360	55.6	33.1		60.0	56.7	4,280	58.5	37.3	7,320	58.7	33.2	4,400	60.4	38.4	5,505	57.9
	D	26.8	3,800	55.9	14.5		60.7	55.8	7,080	58.6	34.6	7,640	57.9	32.6	5,840	59.5	39.9	6,090	58.4
Trumbull	A	16.2	2,440	55.6	26.8		58.7	40.3	2,180	56.5	20.2	2,140	54.6	10.6	1,340	59.4	18.1	2,025	57.7
	B	21.1	2,940	55.4	34.3		59.2	54.8	4,200	58.2	34.6	4,620	57.0	27.3	2,720	59.2	30.0	3,620	57.1
	C	23.8	3,040	54.8	43.9		60.5	56.6	5,040	59.6	39.4	6,820	56.5	36.1	4,280	53.2	37.7	4,945	57.4
	D	20.1	3,660	56.2	11.2		59.3	49.6	5,480	59.6	32.0	7,420	58.5	6.6	5,380	—	38.1	5,485	56.5
Fulhio	A	16.0	2,280	55.9	19.7		60.4	47.7	2,500	56.9	19.2	2,340	58.6	6.6	980	58.4	16.5	2,025	57.8
	B	19.9	3,520	53.0	32.0		61.2	57.1	5,360	56.0	29.9	5,520	56.5	25.3	2,460	59.2	28.3	3,675	57.8
	C	23.0	2,580	52.9	43.8		59.0	59.8	4,140	57.0	35.1	6,760	58.2	38.5	4,200	56.3	37.1	4,960	58.7
	D	24.4	4,080	54.3	15.8		51.7	27.8	6,980	57.3	32.0	7,420	59.6	43.7	5,700	59.3	40.7	6,045	54.6
T. N. 1049 (Portage × Gladden)	A	14.9	2,420	54.3	15.8		57.1	39.7	2,140	56.3	20.7	1,880	56.6	7.3	1,080	55.4	15.1	1,880	56.7
	B	18.5	2,840	54.2	30.0		59.2	53.6	3,920	56.3	33.2	4,540	56.9	21.3	2,880	59.3	25.1	3,470	57.2
	C	22.0	3,520	52.8	35.4		60.5	52.2	5,420	55.4	40.5	6,360	57.0	30.6	4,540	60.4	35.3	4,860	56.3
	D	21.8	4,100	54.2	16.5		55.8	29.1	7,280	55.7	36.9	6,880	57.3	31.8	5,520	59.2	35.5	5,945	55.3
T. N. 1006 (Portage × Fulcaster)	A	14.8	2,680	54.6	16.5		57.1	43.8	2,060	55.8	27.5	2,560	56.3	8.4	1,040	—	17.0	1,970	57.0
	B	19.1	3,660	53.7	28.1		59.2	54.6	4,580	54.4	35.4	6,120	56.3	26.6	2,660	55.4	28.3	3,880	57.5
	C	24.3	4,060	53.2	40.1		59.9	64.0	6,500	55.7	37.2	7,880	55.4	33.5	4,460	59.3	36.1	5,145	57.1
	D	25.2	4,080	55.1	3.3		55.3	26.2	1,700	56.0	27.8	8,280	55.7	45.0	5,720	59.2	40.4	6,140	54.5
T. N. 1034 (Velvet Gipsy × Gladden)	A	13.9	2,080	54.7	13.6		56.6	41.3	3,500	55.4	22.9	2,040	55.8	7.2	1,080	58.4	14.7	1,725	56.5
	B	20.9	2,660	54.1	17.4		58.6	53.1	5,220	55.7	38.5	4,620	54.5	23.1	2,380	—	27.5	3,290	56.5
	C	28.5	3,480	52.7	36.8		59.9	56.9	7,020	55.8	41.9	6,900	53.8	32.1	4,100	55.4	34.6	4,925	55.6
	D	30.2	4,580	52.8	2.3		44.0	25.9	1,820	54.4	36.8	7,520	55.9	32.1	5,680	58.7	38.7	6,200	52.6
Junior No. 6	A	10.9	1,920	52.8	7.9		57.1	44.7	3,840	55.7	14.6	2,000	57.5	3.4	540	54.6	11.4	1,570	55.3
	B	16.6	2,400	55.0	20.8		57.9	53.9	4,820	56.0	33.1	4,090	57.3	19.0	1,600	58.4	24.3	2,980	56.3
	C	23.5	3,360	55.4	34.1		59.2	56.0	7,260	55.4	30.6	4,560	58.1	27.5	3,140	59.1	31.3	4,470	57.1
	D	24.2	4,320								36.1	7,020		38.7	4,660	58.9	37.8	5,815	57.4

T. N. 1045 (Portage × Gladden)	A	10.3	2,100	53.5	9.1	54.2	29.5	2,200	57.4	18.1	2,740	58.1	6.4	1,020	56.6	14.7	2,015	56.0
	B	17.9	2,140	56.2	17.2	59.1	45.4	4,040	58.4	31.4	5,040	58.5	24.3	2,740	60.9	27.2	3,490	58.6
	C	22.8	3,700	56.7	29.5	61.2	57.2	5,780	57.5	38.4	6,720	57.9	33.4	4,160	61.1	36.3	5,090	58.9
	D	29.9	4,660	56.4	41.7	61.8	54.3	7,360	57.3	34.4	7,820	57.9	40.4	5,540	—	40.1	6,345	² 58.4
Average	A	15.3	2,280	54.9	7.5	53.2	27.9	2,140	56.6	19.6	2,200	56.3	6.3	960	54.9	15.3	1,895	55.2
	B	20.8	2,720	55.8	16.8	57.8	43.3	3,900	57.1	32.5	4,800	57.6	23.3	2,460	59.4	27.3	3,470	57.5
	C	26.3	3,680	56.0	27.7	59.5	54.3	5,140	57.5	37.5	6,700	57.5	32.6	4,020	59.6	35.7	4,885	58.0
	D	27.4	4,220	55.8	39.1	60.5	56.1	6,800	56.0	33.2	7,480	57.2	37.7	5,440	³ 59.2	38.7	5,985	57.6

¹ T. N. indicates the test number under which new lines are carried at Wooster.

² 4-year average only.

³ Average of 6 varieties only.

THE 1929-30 CROP

The fall of 1929 was more favorable than that of 1928, especially in respect to moisture relations, and wheat made much better growth. Spring rainfall in 1930 was light but apparently sufficient for a marked response to fertilizer treatments. The untreated plots on level A gave an even smaller crop than in the previous year, but level D yielded 31.6 bushels more grain per acre than did level A. Straw yields were not available for this crop. Level A gave the lowest and level C the highest weight per bushel found over the entire 5-year period.

THE 1930-31 CROP

The season of 1930-31 was the most favorable encountered and appeared to approach ideal conditions for winter wheat. There was excellent fall growth, a dry winter season, and sufficient spring rainfall to give maximum response to the plant nutrients added. The winter was mild and the spring early. Even level A produced a fair crop; in fact, it yielded more grain than did level D in 1929.

In 1931 level D gave 28.2 bushels of grain and 4,660 pounds of straw per acre more than level A. Grain yield followed the general trend of the total yield quite closely, giving a small uniform increase in the straw to grain ratio from level A to level D. Weight per bushel dropped abruptly from level C to level D.

THE 1931-32 CROP

The yields in 1932 were most erratic. The fall of 1931 was exceptionally mild and wheat made phenomenal growth. The coldest month of the winter was March, when wheat normally resumes its active growth. A severe and sudden frost early in the month killed back the tops considerably and another cold snap later caused slight injury after growth had begun. A third frost occurred at heading time. Either this last frost or the very dry soil conditions then prevailing, or a combination of these factors, resulted in some blasting of the heads. This may partially account for the lower grain yields on level D, since this series heads later than the others. It can hardly be the whole explanation, however, since the dates of heading of levels C and D overlap and level D was consistently low in grain yield in 1932.

The total yield in 1932 followed that of 1931 very closely, except for a decidedly smaller increase for level D over level C. Grain yields, however, behaved very differently. Twelve of the sixteen varieties grown gave less wheat on level D than on level C. Level C yielded 17.9 bushels of grain and 4,500 pounds of straw more per acre than level A. Level D gave 5,280 pounds of straw more than level A. Straw to grain ratio was, of course, high on level C and reached 3.76 on level D. A very high proportion of the dry matter in the total yield of level D was recovered in the straw, as compared with that recovered in other seasons. No study of the heads was made, so that it was not determined whether the lower grain yield was due to fewer heads, fewer kernels per head, or poorer filling of the kernels. The weight per bushel would not indicate shriveled grain. There were no significant differences in length of straw and no lodging on levels C and D.

A more normal crop season occurred in 1932–33. During February there was one cold snap with subzero temperature, but this caused no damage except to tops. Fall conditions were favorable, winter precipitation light, and early spring rainfall probably excessive, followed by a dry period from the middle of May to harvest time. It probably was due to this dry spell and also to the high March precipitation, which may have caused some leaching of nitrates, that yields were reduced as compared to those of 1931.

In 1933, in both total yield and grain yield, the performance paralleled very closely that of 1931, except that all yields were distinctly lower. Level D gave 31.4 bushels of grain and 4,480 pounds of straw per acre more than level A, indicating marked response even to heavy fertilizer applications. With the exception of level A, on which yields were very low, weight per bushel was high and varied little from level to level.

ANALYSIS OF VARIANCE

From a general consideration of the data, it was evident that seasonal factors operating in Ohio influenced yield markedly. The differences between the yearly averages were greater than those between varieties in any one season. Varietal response, however, also appeared to be a factor. To test the validity of this assumption and to obtain some idea of the significance of the variations noted, analyses of variance were made, according to the method developed by Fisher (1), first for total yields (that is, the weight of the sheaves before threshing) and again on the grain yields. To estimate the odds for significance, the direct ratio of the variances, Snedecor's F (7) was used in place of Fisher's Z.

The results of the analysis of total yield for individual seasons are presented in the first part of table 3. Four years' data only were available. Variety, level, replication, and the variety-level interaction were considered. The analysis was made from the yield in pounds per plot and the figures were rounded off to the nearest pound.

In both total yield and grain yield some plot yields were missing. These were compensated for by using the actual number of plots in each group when dividing the sums of squares. For example, in the total yield for 1930–31, 2 of the 11 varieties were sown in only two replications. In calculating the variance due to variety, nine variety sums were squared, added, and divided by 12, while the other two variety sum squares were added and divided by eight. The two mean values thus obtained were added to give the total sum of squares for variety.

The combination of poor season and lack of response to treatments already discussed had a marked effect on the 1929 results. F values for that year are low because of the small differences between high- and low-level plots, and not because of an abnormally high unaccounted-for error. Even in this season variance due to variety, level, and replication was significant.

In all seasons there were significant differences between varieties, and very significant differences between levels, and between replications, excepting only in 1928–29. The variety-level interaction was significant only in 1930–31, the most favorable season. This suggests

TABLE 3.—*Analysis of variance, by seasons, of total yield and for grain yield for 11 varieties of winter wheat grown at four soil fertility levels in four and five seasons*

TOTAL YIELD

Season	Variance due to—	Degrees of freedom	Sums of squares	Variance	F	1-percent point for F	5-percent point for F	
1928–29	Variety	10	209	20. 9	2. 71	2. 72	2. 04	$\bar{x}=19.7$
	Level	3	2, 795	931. 7	121. 00	4. 01	2. 71	$\sigma=2.77$
	Replication	2	51	25. 5	3. 31	4. 85	3. 10	C. V. = 14. 1%
	Variety-level interaction	30	219	7. 3	. 95	2. 00	1. 64	
	Error	86	658	7. 7				
	Total	131	3, 932					
1930–31	Variety	10	214	21. 4	3. 89	2. 74	2. 06	$\bar{x}=30.9$
	Level	3	12, 407	4, 135. 7	751. 95	4. 04	2. 72	$\sigma=2.35$
	Replication	2	144	72. 0	13. 09	4. 88	3. 11	C. V. = 7. 6%
	Variety-level interaction	30	320	10. 7	1. 95	2. 03	1. 65	
	Error	78	431	5. 5				
	Total	123	13, 516					
1931–32	Variety	10	566	56. 6	5. 24	2. 72	2. 04	$\bar{x}=30.6$
	Level	3	14, 169	4, 723. 0	437. 31	4. 01	2. 71	$\sigma=3.29$
	Replication	2	298	149. 0	13. 80	4. 85	3. 10	C. V. = 10. 8%
	Variety-level interaction	30	146	4. 9	. 45	2. 00	1. 64	
	Error	86	931	10. 8				
	Total	131	16, 110					
1932–33	Variety	10	578	57. 8	8. 38	2. 72	2. 04	$\bar{x}=20.3$
	Level	3	13, 599	4, 533. 0	656. 96	4. 01	2. 71	$\sigma=2.63$
	Replication	2	174	87. 0	12. 61	4. 85	3. 10	C. V. = 13. 0%
	Variety-level interaction	30	198	6. 6	. 96	2. 00	1. 64	
	Error	86	595	6. 9				
	Total	131	15, 144					

GRAIN YIELD

Season	Variance due to—	Degrees of freedom	Sums of squares	Variance	F	1-percent point for F	5-percent point for F	
1928–29	Variety	10	1, 086	108. 6	6. 58	2. 58	1. 97	$\bar{x}=22.5$
	Level	3	3, 088	1, 027. 0	62. 24	4. 04	2. 72	$\sigma=4.06$
	Replication	2	310	155. 0	9. 39	4. 88	3. 11	C. V. = 18. 0%
	Variety-level interaction	30	549	18. 3	1. 11	2. 02	1. 64	
	Error	82	1, 349	16. 5				
	Total	127	6, 382					
1929–30	Variety	10	2, 788	278. 8	20. 96	2. 59	1. 98	$\bar{x}=22.1$
	Level	3	16, 254	5, 418. 0	407. 37	4. 07	2. 74	$\sigma=3.65$
	Replication	2	401	200. 5	15. 08	4. 92	3. 13	C. V. = 16. 5%
	Variety-level interaction	30	646	21. 5	1. 62	2. 07	1. 66	
	Error	72	956	13. 3				
	Total	117	21, 045					
1930–31	Variety	10	1, 049	104. 9	4. 20	2. 58	1. 97	$\bar{x}=45.4$
	Level	3	15, 918	5, 306. 0	212. 24	4. 04	2. 72	$\sigma=5.00$
	Replication	2	1, 100	550. 0	22. 00	4. 88	3. 11	C. V. = 11. 0%
	Variety-level interaction	30	675	22. 5	. 90	2. 02	1. 64	
	Error	78	1, 947	25. 0				
	Total	123	20, 689					
1931–32	Variety	10	687	68. 7	2. 48	2. 58	1. 97	$\bar{x}=31.0$
	Level	3	5, 422	1, 807. 3	65. 25	4. 04	2. 72	$\sigma=5.24$
	Replication	2	347	173. 5	6. 26	4. 88	3. 11	C. V. = 16. 9%
	Variety-level interaction	30	752	25. 1	. 91	2. 02	1. 64	
	Error	77	2, 132	27. 7				
	Total	122	9, 340					
1932–33	Variety	10	1, 240	124. 0	7. 95	2. 55	1. 95	$\bar{x}=24.9$
	Level	3	19, 003	6, 334. 3	406. 04	4. 02	2. 71	$\sigma=3.95$
	Replication	2	237	118. 5	7. 60	4. 86	3. 10	C. V. = 15. 9%
	Variety-level interaction	30	511	17. 0	1. 09	1. 96	1. 60	
	Error	85	1, 330	15. 6				
	Total	130	22, 321					

that as conditions approach the ideal, varietal response to level becomes a more and more important consideration. Evidence for this is not entirely convincing, however.

In the second part of table 3 is presented a similar analysis for grain yields. Five seasons' results were available. Plot yields to the nearest bushel per acre were used as a basis for this study. Two plots gave less than 0.5 bushel and in these cases a yield of 0 was given and included in the n values.

The 1928–29 results, as was the case with total yields, are less significant than are those of other seasons. Variety-level interaction is in no case significant and shows little uniformity from season to season. If any relationship exists it must be complex, for there is poor agreement between the significance of the interaction for total yields and for grain yields in any season.

The generally lower values for F in the second part of table 3 are due in large measure to the relatively larger unaccounted-for error, as is evident from the larger coefficients of variability. This would indicate factors influencing the filling of the grain which did not affect total yield, or at least not to as great an extent. Threshing errors were probably responsible for part, but certainly not for all this increased error.

In total yield was represented the complete dry matter of the plant, while in the grain was found only that portion translocated during the filling period. The amount and rate of this translocation was dependent, in part at least, upon season and was restricted to a definite and rather short period in the life history of the wheat plant. Since level of fertility influenced dates of heading and ripening, even in one season varieties may have differed in environment for heading, filling, and ripening. Greater variability was, therefore, to be expected in grain yields than in total yields. In other words, there were factors operating which determined whether the dry matter synthesized by the wheat plant was recovered in the straw or in the grain at harvest time.

The data were analyzed, all seasons together, for total yield and also for grain yield. When these analyses were made from the individual plot yields, variance due to replication could not be removed because three different soil blocks had been used. This materially increased the unaccounted for error and resulted in lower statistical significance. By using the mean yields of the three replicates of each variety at each level in each season as a starting point, variance due to replication did not enter into the results, and the difficulty was in part overcome. The fact that the variance due to variety, level, season, and the three interactions as presented in table 4 is roughly one-third that obtained when the calculations were made from individual plot yields indicates that no serious error was introduced by using the mean values. The analyses presented in table 4 are thus justified.

From table 4 it is seen that insofar as total yield is concerned, varieties respond differently to level of fertility and to season. This table shows that these interactions are also significant when grain yields alone are considered.

TABLE 4.—*Analysis of variance for total yield and grain yield of 11 varieties of winter wheat grown at 4 levels of soil fertility during 4 and 5 seasons*

TOTAL YIELD (4 SEASONS)

Variance due to -	Degrees of freedom	Sums of squares	Mean square (variance)	F	1-percent point for F
Variety	10	241.01	24.10	15.55	2.55
Level	3	13,510.75	4,503.58	2,905.53	4.01
Season	3	5,130.97	1,710.32	1,103.43	4.01
Variety-level interaction	30	139.94	4.66	3.01	2.00
Variety-season interaction	30	293.72	9.79	6.32	2.00
Level-season interaction	9	1,259.08	139.96	90.30	2.66
Total accounted for	85	20,576.07			
Error	90	139.88	1.55		
Total for experiment	175	20,715.95			

$\bar{x}=25.416$ pounds per plot
$\sigma=1.24$ pounds
C. V. =4.88 percent

GRAIN YIELD (5 SEASONS)

Variety	10	1,204.68	120.47	18.59	2.49
Level	3	18,081.27	6,027.09	930.10	3.94
Season	4	16,336.56	4,084.14	630.26	3.47
Variety-level interaction	30	348.23	11.61	1.79	[1] 1.94
Variety-season interaction	40	1,316.14	32.90	5.08	1.94
Level-season interaction	12	2,924.82	243.74	37.61	2.33
Total accounted for	99	40,211.70			
Error	120	777.68	6.48		
Total for experiment	219	40,989.38			

$\bar{x}=29.3$ bushels per acre
$\sigma=2.55$ bushels
C. V. =8.70 percent

[1] 5-percent point for $F=1.60$.

DIRECT COMPARISON OF VARIETIES

The primary reason for establishing the fertility-levels experiment was to determine whether or not varieties differed from one another in their response on poor as compared with rich soils and particularly to obtain information on the reaction of new lines that the station was developing. A large proportion of the varieties and strains included were new selections. The wheats grown were all reasonably well adapted to conditions in Ohio, and, therefore, the significance of the differences cannot be attributed to the inclusion of some varieties which could not react normally at Wooster. The analyses of variance indicated, however, that real differences in the behavior of these varieties did exist. It will be interesting, therefore, to compare the performance of one of the highest yielding Ohio lines, T. N. 1006 (Portage × Fulcaster), and that of Michikof, an Indiana variety. The data are presented graphically in figure 3. The actual figures are found in table 2.

In total yield the same general trend is noted in both varieties. For the 1929 crop the two curves are very similar. In the other three seasons for which data are available, T. N. 1006 appeared to be able to make better growth than Michikof at the higher levels of fertility, and the differences became greater as the available nutrients increased.

This is the type of differential response of variety to level which apparently accounts for the significant interaction of these factors.

For the grain yields, very similar relationships held. In the crops harvested 1930, 1931, and 1933, T. N. 1006 gave greater response to fertilizer in grain and in total yield as well in the last two of these seasons. Total yields were not available for 1930. In four crops out
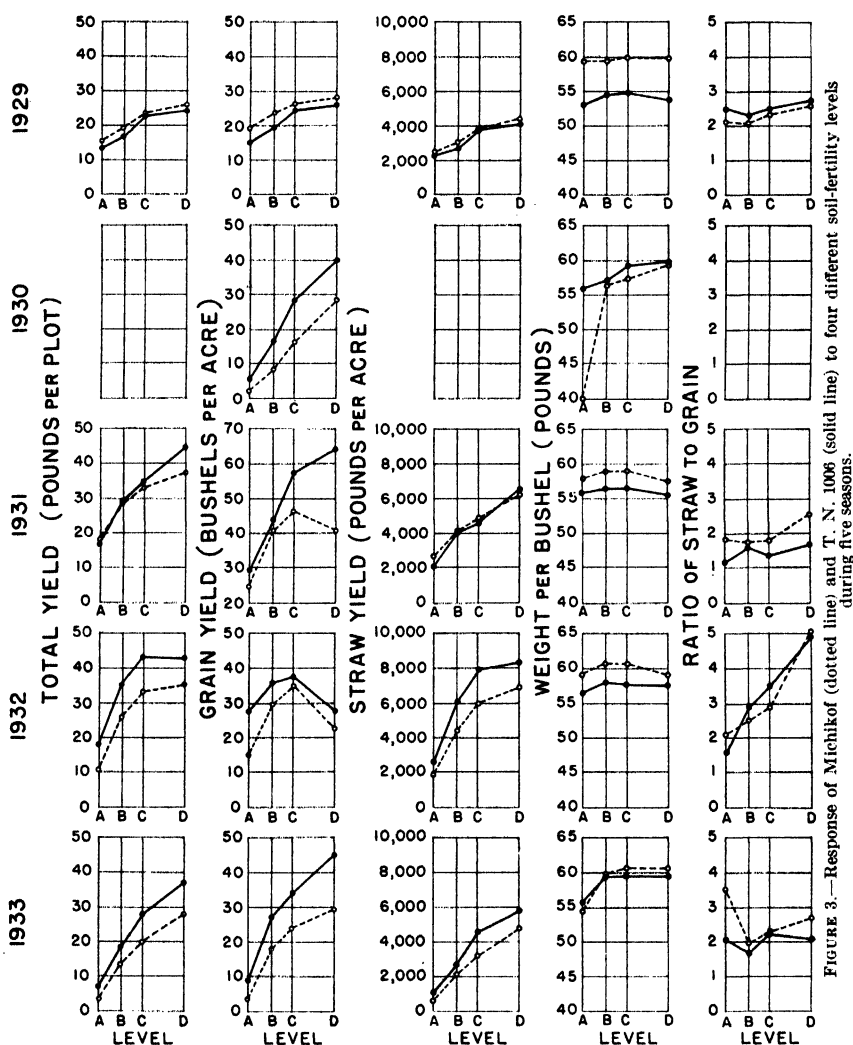
FIGURE 3.—Response of Michikof (dotted line) and T. N. 1006 (solid line) to four different soil-fertility levels during five seasons.

of the five, T. N. 1006 gave a higher grain yield than Michikof at every level. However, in 1929 Michikof consistently produced more grain. It may be significant that this last-mentioned season was the one in which fertilizers had the least effect.

In 1931 T.N. 1006 demonstrated its ability to give very high yields when conditions are suitable. Michikof in the same season dropped in grain yield from level C to level D in all three replications. This

probably indicates a real difference in ability to utilize large amounts of plant nutrients for grain production.

Michikof is a rather hard wheat; T. N. 1006 is soft. In general, hard wheats have not yielded so well as soft at Wooster. Whether there is a connection between class of wheat and response to fertilizer could not be determined from the data at hand.

Michikof showed less variation in weight per bushel than did T. N. 1006, and it was generally heavier. The extremely low yield of Michikof at level A in 1930 and 1933 was undoubtedly the reason for the abnormal bushel weights on these plots. Both wheats followed the average trend and reached a maximum at about level C. Weight per bushel was apparently subject to greater variation in T. N. 1006 than in Michikof, especially between seasons.

SUMMARY AND CONCLUSIONS

A 3-year rotation of corn, oats, and winter wheat has been grown at four fertility levels for five seasons at the Ohio Agricultural Experiment Station. Wheat alone is considered in this paper.

Data on grain yield, straw yield, and weight per bushel are given for 11 wheat varieties which were grown in all five seasons.

Climatological data for the period covering the five wheat crops are presented.

Analysis of variance of total yield and of grain yield indicates that differences due to variety, to fertility level, to season, and to each of the three interactions of these factors cannot be ascribed to errors of random sampling alone. In all but one case the odds for significance exceed 99:1, when all seasons are considered together.

In the analysis of variance, residual error for the grain yields is greater than that for total yields, as shown by the coefficients of variability; this indicates that factors other than those considered influence translocation appreciably. Date of heading and of maturity probably fall in this class, since they result in variations in the environment during the period in which the grain is filling and hardening.

The results obtained indicate definitely that varieties respond differently to a series of fertility levels. Of the varieties used, none was definitely superior at only the high or at only the low levels of fertility. The data, however, do not preclude the existence of such varieties. Further work is being conducted in an attempt to analyze the response to fertility levels into its components.

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ROOT GROWTH OF SEEDLINGS OF PINUS ECHINATA AND PINUS TAEDA [1]

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INTRODUCTION

The operations of growing and transplanting southern pine seedlings for reforestation and afforestation purposes are still beset with the danger of high mortality of transplanted stock. The advantages of fall over spring transplanting, or vice versa, are yet to be determined, and, of course, will be determined only after many years of actual planting in both seasons. In the Southern States where winter growth, or merely semidormancy, of at least some plant parts is apt to obtain, it seems possible that the performance of roots, as regards duration or seasonal periodicity of growth, may be an important factor influencing mortality. The object of this experiment was to investigate the growth of roots of shortleaf and loblolly pine seedlings, with particular reference to the extent and time of elongation.

REVIEW OF LITERATURE

A comprehensive review of the literature pertaining to root growth has been given by Stevens (7).[2] However, a few references not included in his publication may be cited.

Petersen's work (5) supports the conclusions of others that there may be some degree of winter growth of roots of deciduous trees. Schimper's observations (6) led him to conclude that there is periodicity in plant growth, which he attributed to causes within the plant. Engler (1) believed that there occur two periods of tree-root elongation, one in the spring, the other in the fall. Brown (2) inferred that root growth of *Pinus strobus* ceased in autumn and was resumed in April. MacDougal (4), referring to tree growth in general, stated that although continuous cambial activity may be expected in evergreen trees, growth ceased in Monterey pine when the cambial temperature fell below 8° C. Kinman (3) observed no winter growth of roots in the case of mature myrobalan, peach, and apricot trees. He found two periods of more active root growth: In February and March, for both myrobalan and peach trees, from mid-September to mid-November for myrobalan, and from mid-October to mid-November for peach, with essential cessation between these periods. The apricot had one wholly inactive period, from late January to late March.

METHOD OF STUDY

Two frames of truncated-wedge shape (fig. 1) were constructed and placed in excavations at the Arkansas Agricultural Experiment Station at Fayetteville. The top of each frame was at the level of the surface of the ground. The sides of the frames were inclined

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inward from the base at an angle of approximately 18° from the vertical. Each side of a frame accommodated ten 10-inch by 24-inch pieces of double-strength glass[3] which were supported by the base, top, and vertical braces. Hinged, lightproof and rainproof lids were constructed to divert rain water into ditches which led away from the frames, and tile was placed under each box to take off seepage water during rainy periods. Pulverized A_1 horizon Clarksville silt loam was placed around the frames and allowed to settle three times with subsequent refilling. Care was taken at all times to keep the soil layer above the level of the glass to exclude light.

On April 1, 1933, twenty 1-year-old seedlings each of *Pinus echinata* Mill. (shortleaf pine) and *P. taeda* L. (loblolly pine) were planted along the sides of the frames with roots against the glass. On April 26, 1933, the exact position and length of all roots visible was etched
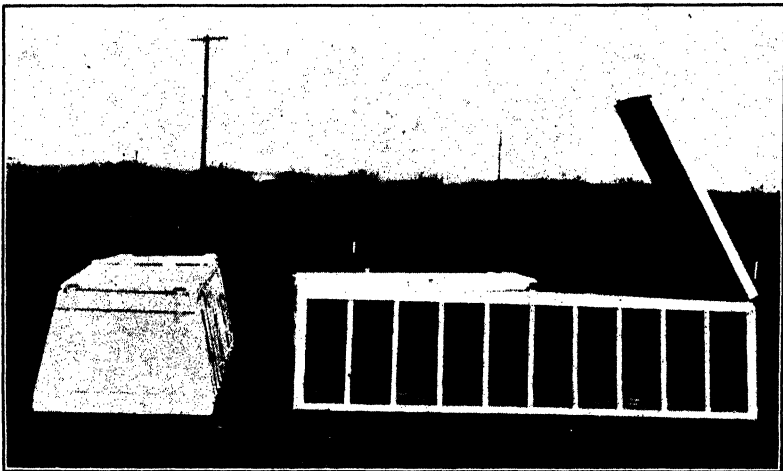
FIGURE 1.—Root-growth observation frames before installation.

on the interior of the glass with a diamond-pointed pencil. Subsequently, at intervals averaging 8 days, etchings were made of all active roots, with numbers referring to recorded dates, throughout a period of 2 years. At the end of that time the glasses were removed, marked in three vertical intervals of 8 inches each, and the extent of root growth in each of these intervals was determined for the periods of observation. All readings were reduced to daily average growth in length in millimeters for each level for each period. In the records no attempt was made to separate horizontal from vertical growth of roots.

The mean temperature in Fahrenheit and the precipitation for each observation period were determined from the records of the meteorological station situated 600 feet from the frames.

RESULTS

The observed occurrence of continuous growth (fig. 2) during the entire period of 2 years, in at least two levels, is particularly sig-

[3] Six glasses were broken by the pressure of the growing roots. Stronger glass or narrower pieces would doubtless have proved more satisfactory.
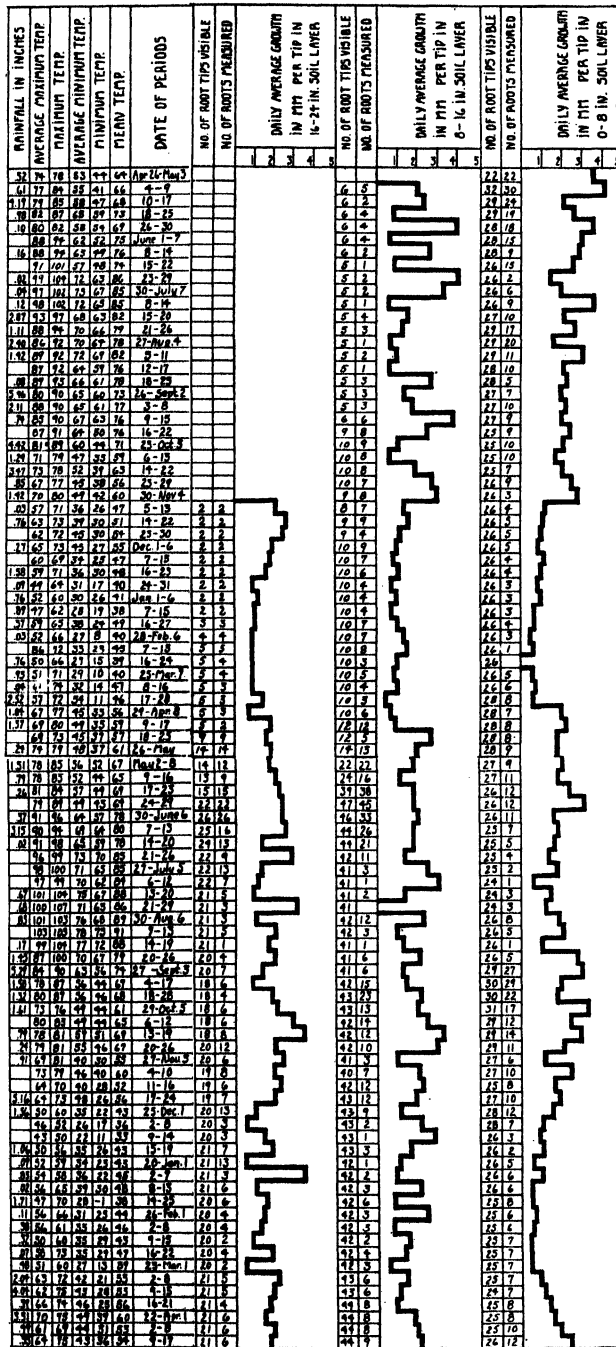
FIGURE 2.—Root growth of *Pin₁₅ echinata* seedlings from April 26, 1933, to April 17, 1935.

nificant.[4] The absence of records of growth for the upper level for the period of February 16–24, 1934, and for the middle layer for the period of July 21–29, 1934, is probably fortuitous. The fact that the roots of the young seedlings had not yet reached the lowest level accounts for the absence of records in that level between April 26, and November 4, 1933.

Periods occurred in which there were both greater average daily increment to each root and a larger number of growing roots. These were: Late April to early June of 1933, mid-July to mid-August of 1933, early April to early June of 1934, early September to late October of 1934, and early April of 1935. All of these periods of greater growth were coincident with a mean temperature of 55° F. or higher, and with the occurrence of considerable rainfall.

Likewise, periods occurred in which there was relatively little growth. During some of these there was both little daily average increment and a small number of active roots. These periods were: Early November to mid-March of 1933–34, mid-June to mid-August of 1934, and early December to late March of 1934–35. The first- and third-named periods were coincident with mean temperature of 56° F. or lower, and the second with low rainfall. One period, from early June to mid-July of 1933, with low rainfall, showed a conspicuous diminution in the number of active roots, but a less conspicuous lessening of daily average increment. There is evidence of correlation of least number of active roots and lowest daily average increment with periods of lowest average minimum temperature occurring during the 2 years, that is, from November 5, 1933, to March 28, 1934, and from November 11, 1934, to March 1, 1935.

With the resumption of greater activity in the spring there was a noticeable lag both in average daily increment and in the number of active roots in the lower levels as compared to the top.

There is some evidence that prolonged dry periods have a greater retarding effect on root growth in the upper level than in the lower levels. This is observable in the periods of June 1933, and mid-June to mid-July of 1934. In general, the effects of both low temperature and low rainfall were more evident in the topsoil layer than in the lower ones. Conspicuous fluctuations in daily average increment occurred in the middle layer in 1933, but after that the roots in both of the lower levels showed less tendency to vary, at least as regards rate of growth, than those of the top layer. During the winter, or the time when the mean temperature of periods was 53° F. or lower, high rainfall had no accelerating effect on rate of root growth in any of the levels.

DISCUSSION OF RESULTS

The period of greater root activity in the spring is naturally associated with the various phenomena and conditions attending the end of the semidormant winter period and the beginning of the period of active stem growth. A second period of semidormancy occurred during the very warm and dry summer months, followed by a renewal of active growth during the cooler, rainy period of early autumn. It should be mentioned that in both years there was a noticeable recurrence of stem growth during this latter period.

[4] Graphic and tabular representation of growth is for *Pinus echinata* only. There was not enough difference in performance of roots of *P. taeda* to justify separate presentation.

The greater number of records of active roots during or following rainy periods is due both to the fact that new roots are initiated and that inactive roots become active again at such times. The retardation of activity in the top layer during extremely hot, dry weather may be due to reduced soil moisture, and possibly to high, unfavorable temperature of the soil.

Within the limits of the research method, the evidence secured supports the opinion of some earlier investigators that root growth of trees is slow in summer as a result of low moisture content of the soil; in winter due to low temperature; and greater during periods of high soil moisture and favorable temperature. It would probably be an error, however, to wholly dissociate the slow growth, or semidormancy of roots in midsummer from other factors, such as high transpiration, and probably excessively high temperatures of the topsoil layer.

SUMMARY

Roots of seedlings of *Pinus echinata* Mill. and *P. taeda* L. made recordable growth during every 8-day period for 2 years.

Periods of more active growth were observable—in early spring, and late summer-early autumn, and in 1 year, in midautumn.

There were two marked periods of semidormancy; both the number of growing roots and the daily average increment were lower in the period from December 1 to March, and from the end of June through August.

The periods of slow growth, winter and midsummer, were associated with low air temperature and low rainfall, respectively. Greatest activity of roots was associated with considerable rainfall and favorable but not too high air temperature.

There was less growth in the top 8-inch layer than in lower levels during periods of lowest air temperature, and of high air temperature and low rainfall. In general, there was somewhat less variation in the rate of growth of roots in the two lower levels than in the upper one.

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INHERITANCE OF COMPLEMENTARY DWARFING FACTORS IN WHEAT [1]

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INTRODUCTION

The occurrence of dwarfs in cereal and other plant hybrid populations is being reported by an increasing number of investigators. In most cases in the cereals it has been observed in connection with hybridization experiments for economic improvement. The crosses reported in this paper were produced incidentally in a wheat-breeding program, the main object of which was the production of improved bunt-resistant varieties. Complete dwarfing occurred in the F_1 generation of eight crosses, although dwarfs more commonly are found as segregates in the F_2 generation.

The dwarf condition in plants apparently is due to genetic factors, to chromosome abnormalities, or to other causes that interfere with normal height development. It is now generally recognized that dwarfing of the kind usually encountered in intraspecific hybrids is governed chiefly by factor interactions but that the ratios obtained usually are modified to some extent by irregular chromosome behavior.

The presence of dwarfs in wheat crosses which first appear in the F_2 generation has been explained by a number of investigators by the interaction of two factors, a factor for normal height, N (or I), which when present inhibits the dwarfing factor, D. In these experiments the F_2 segregation occurred in a ratio of 13 normal to 3 dwarf plants. Workers whose results with dwarfing have been explained on a two-factor basis include Hayes and Aamodt (6),[2] Clark and Hooker (2), Goulden (5), Stephens (9), Stewart and Tingey (10), Clark and Quisenberry (3), Nieves (8), Churchward (1), Tingey (12), and Waterhouse (14).

Other workers, viz, Waldron (13), Neethling (7), Florell (4), and Churchward (1), obtained segregations that indicated either three- or four-factor differences but which possibly are best explained as poor fits to the two-factor difference obtained by other workers.

Dwarfs in the F_1 generation from normal parents have been observed by a number of wheat breeders, including Waterhouse (14), who found dwarfs in the F_1 generation in 25 wheat crosses. Results from such dwarfs carried through the F_2 and F_3 generations were presented by Thompson (11).

These results were explained on the basis of the interaction of three factors. Thompson assumed the presence of the usual factor for normal, or inhibitor for dwarf, I (or N), with the dwarfing factor (D) and an extra inhibitor (E), which inhibited or neutralized the action

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of the inhibitor I. Thus, the dwarf plants were conditioned by the genes IED and ieD, and the normal plants by IeD and IEd.

According to Thompson's scheme the F_2 population will segregate in a ratio of 39 D : 25 N. The F_2 normal plants selected at random will produce F_3 families in a ratio of 19 true-breeding normal to 6 segregating normal and dwarf, and the F_2 dwarf plants will produce 7 true-breeding dwarf families to 32 segregating dwarf and normal.

MATERIAL AND METHODS

More than 50 crosses were made at the Idaho Agricultural Experiment Station, Moscow, Idaho, in 1931, by the senior writer and his assistants in a wheat-breeding program designed to develop improved bunt-resistant varieties of white winter wheat for the Pacific coast region. Four of these crosses and their reciprocals produced all dwarf plants in the F_1 generation. Turkey-Florence (G–326W8) was a parent in three of these crosses and Hussar-Hohenheimer (C. I. 10068 [3]) in the other. Dwarfs in the F_1 generation also were obtained in 1933 from two Arco selections (8118 and 8120) crossed with both Turkey-Florence and Hussar-Hohenheimer in 1932. Some F_1 plants of the Hussar-Hohenheimer $\times$ Jenkin cross were backcrossed to both parents in 1932.

Turkey-Florence is a white-kerneled, bunt-resistant winter wheat selected from the same cross from which Ridit was produced. The original Turkey-Florence cross was made by E. F. Gaines at the Washington Agricultural Experiment Station, Pullman, Wash., and the white-kerneled Turkey-Florence selection used in the present investigation was made by D. E. Stephens at Moro, Oreg.

Hussar-Hohenheimer (C. I. 10068) was selected by the late H. M. Woolman at Corvallis, Oreg. This wheat is a bearded, red-kerneled, white-strawed strain having a winter habit of growth.

Jenkin is a late-maturing spring variety of club wheat having tall white straw, soft white kernels, and brown chaff. Federation is a medium early spring variety having short stiff straw, soft white kernels, and brown chaff. Baart is an early spring variety having medium-tall, slender, and pliable straw and large white semihard kernels.

The Arco selections were obtained from the cross Arcadian $\times$ Hard Federation, which was made by Walter Carpenter at Moro, Oreg., in 1919. The Arco selections have short stiff straw, soft white kernels, and a winter habit with rather low winter hardiness.

Dwarf plants usually can be distinguished readily from normal plants by the much reduced height and by the abundance of grassy leaves at the base of the plants resulting from their shortened internodes. Height measurements of the F_1 and F_2 dwarfs and the parents were taken for comparison, but in the F_3 generation the dwarfs were distinguished by inspection only.

In making the crosses, pollen from a single spike was used to fertilize each emasculated spike. A good set of seed was obtained both in 1931 and 1932. The crossed kernels were sown 4 inches apart in 5-foot rows spaced 1 foot apart, between a row of each of the parents. Fairly vigorous F_1 hybrid plants were obtained in both 1932 and 1933,

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

although there was some winter-killing of tender strains in both years. In 1932 Baart winter-killed, and a somewhat reduced stand was obtained in both the Federation and Jenkin parents. Good stands were obtained in the F_1 hybrids and vigorous plants were produced in the hybrids as well as in the parents that survived.

The height of each of the hybrid and parent plants studied was obtained by measuring the height of the earliest culm. The length of spike of the earliest culm also was measured and the average weight of kernel in milligrams was determined from the weight of 100 kernels from each plant.

Most of the F_1 plants produced seed fairly abundantly. Dwarf plants that did not produce spikes were designated as grass plants or grassy dwarfs. Seeds from F_1 dwarf and parent plants were sown in late October at the rate of 100 kernels per row in 16-foot rows 1 foot apart. Emergence did not take place before the winter snows began, and fall-stand counts could not be made in 1932.

A good snow covering was present during most of the winter months, but only fair winter survival was obtained. The dwarfs showed most evidence of winter injury. The growing season again was favorable so that vigorous plants were obtained from most of the surviving individuals.

At harvest time, plant height, length of spike, and average weight of kernel per plant were obtained as in the F_1 generation. Seed of good quality again was obtained from most of the dwarfs as well as from the parent plants.

In the fall of 1933, this experiment was transferred to the Pendleton Field Station, Pendleton, Oreg., where the crop was grown and the data were taken by the junior writer. Seed from about 25 to 35 dwarf F_2 plants and from about 35 to 70 normal F_2 plants was selected at random from each of four crosses to continue the experiments in the F_3 generation. About 75 to 100 kernels, in some instances fewer, from each plant were sown in 16-foot rows 1 foot apart. Reasonably satisfactory emergence occurred, but some of the weaker plants were lost during the winter. Limited moisture and hot weather in the spring caused many dwarfs to die prematurely and rendered identification difficult. These conditions also limited the height of some weaker normal plants.

EXPERIMENTAL DATA

THE F_1 GENERATION

The average height of culm, length of spike, and weight of kernel of four dwarf F_1 wheat crosses and of their normal parents, at Moscow, Idaho, in 1932, are presented in table 1. Similar data, except for kernel weights, for four additional crosses, in 1933, are also given.

The average height of the dwarfs in 1932 was about 15 to 17 inches; that of Turkey-Florence 44 inches, of Jenkin 43 inches, and of Federation 33 inches. The standard deviations of the heights of the F_1 hybrids were similar to those of the normal parents but were relatively larger owing to dwarfness of the plants. The mean height of the F_1 dwarfs in 1933 ranged from about 10 to 13 inches. The parents likewise were shorter than in 1932, owing to dry weather at maturity and to poor soil. The F_1 dwarfs and parents of two of these crosses are shown in figure 1.

TABLE 1.—*Average culm height, spike length, and kernel weight of eight dwarf F_1 wheat crosses and their normal parents, at Moscow, Idaho, 1932 and 1933*

Year and parent or hybrid	Plants	Mean and standard deviation		
		Culm height	Spike length	Kernel weight
1932	*Number*	*Inches*	*Cm*	*Mg*
Turkey-Florence	6	45.17± 1.47	11.67±1.92	47.50±1.92
F₁ hybrid	15	16.92±10.40	7.03±2.43	39.77±6.11
Jenkin	9	43.44± 2.87	6.60± .78	45.80±4.40
Turkey-Florence	8	44.63± 2.99	10.94±1.63	46.25±1.66
F₁ hybrid	7	15.43± 2.91	8.50±1.29	37.56±4.32
Federation	11	32.86± 5.45	8.64±1.91	42.73±5.04
Turkey-Florence	6	41.33± 2.83	9.92±1.17	37.00±1.15
F₁ hybrid	7	15.71± 3.57	8.00±1.36	
Baart ¹	0			
Hussar-Hohenheimer	9	39.56± 8.17	11.0'±1.69	45.00±3.49
F¹ hybrid	8	14.88± 7.77	9.81±3.39	
Jenkin	2	38.50±10.59	6.00±1.42	47.00±1.42
1933				
Hussar-Hohenheimer	8	33.38± 2.98	8.94± .47	
F₁ hybrid	10	9.70± 2.12	9.15±1.59	
Arco (sel. 8120)	9	28.56± 3.25	7.67±1.86	
Turkey-Florence	1	36.00	10.00	
F₁ hybrid	1	13.00	9.00	
Arco (sel. 8120)	3	30.00± .00	7.88± .41	
Arco (sel. 8118)	3	32.33± 3.11	8.33± .58	
F₁ hybrid	3	12.00± 4.20	7.67± .82	
Turkey-Florence	4	39.00± 2.35	10.25±1.50	
Arco (sel. 8118)	13	29.13± 3.43	8.00±1.21	
F₁ hybrid	13	10.69± 5.30	8.69± .80	
Hussar-Hohenheimer	12	36.58± 2.22	9.33± 2.48	

¹ Winter-killed.

The length of the main spike of the F_1 dwarfs was intermediate in some crosses but less than that of either parent in others.

The average weight of kernel is given for only two crosses, but in both cases it is definitely less than in either parent.

The first generation of the Hussar-Hohenheimer × Jenkin backcrosses was grown at Moscow, Idaho, in 1933. The proportion of normal to dwarf plants expected in the backcross to either parent was 1:1. Thirty-five mature plants were obtained from the backcross to Hussar-Hohenheimer, of which 17 were dwarf and 18 normal. This was close to the expected result. Seven plants, all normal, were obtained from the backcross to Jenkin, the number of plants apparently being too small to give the expected segregation.

The mean height of the dwarf plants, not including two grassy plants, was 10.47±5.84 inches, and that of the normal plants 32.94± 5.88 inches. The mean height of the Hussar-Hohenheimer parent (28 plants) was 31.14±8.73 inches, and that of the Jenkin parent (4 plants) 32.00±4.69 inches. The mean height of the 7 normal plants obtained by backcrossing the F_1 with Jenkin was 31.86±9.21 inches.

THE F₂ GENERATION

The number of seeds of the different crosses sown and the plants harvested in the F_2 generation were as follows: Turkey-Florence × Jenkin, 688 sown and 394 (57.3 percent) harvested; Turkey-Florence × Federation, 381 sown and 139 (36.5 percent) harvested; Turkey-Florence × Baart, 366 sown and 147 (40.2 percent) harvested; Hussar-Hohenheimer × Jenkin, 410 sown and 137 (33.4 percent) harvested.

It is evident that all of the crosses suffered serious winter injury, since in favorable seasons survivals of over 90 percent are not uncommon. The data on normal and dwarf plants in the F_2 generation are presented in table 2. According to Thompson's three-factor hypothesis, these crosses should have given dwarf and normal plants in a ratio of 39 D:25 N. This ratio was indicated only in Turkey-

FIGURE 1.—Dwarf F_1 hybrid and parental plants at Moscow, Idaho, 1933. In foreground: *a*, Hussar-Hohenheimer; *b*, dwarf hybrid; *c*, Arco; *d*, Turkey-Florence; *e*, dwarf hybrid; *f*, Arco.

Florence × Baart, in which the deviation was 3.3 times the probable error. Only in this cross did the number of dwarfs exceed the number of normal plants.

TABLE 2.—*Number of normal and dwarf plants in the F_2 generation in four wheat crosses at Moscow, Idaho, 1933*

Cross	F_1 families	F_2 plants					
		Total	Observed		Calculated [1]		Deviation
			Normal	Dwarf	Normal	Dwarf	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Turkey-Florence × Jenkin and reciprocal	6	394	245	149	154	240	91±6.5
Turkey-Florence × Federation	4	139	83	56	54	85	29±3.2
Turkey-Florence × Baart	3	147	70	77	57	90	13±4.0
Hussar-Hohenheimer × Jenkin and reciprocal	6	211	137	74	82	129	55±4.8

[1] Based upon the ratio of 39 dwarf to 25 normal.

It seems probable that winter-killing destroyed the greater portion of the dwarf segregates. The number of normal plants did not exceed the number that might be expected from a reasonably good survival, whereas the number of dwarf plants was decidedly less. Dwarf plants are less vigorous than normal plants, and therefore more likely to be injured by unfavorable conditions.

The mean height of the dwarfs in the F_2 generation ranged from 12 to 15 inches. A number of somewhat taller dwarfs, which probably represented heterozygotes, were classified as intermediate dwarfs. These had the typical dwarf characteristics in most respects but were 28 to 34 inches in height. There was little or no overlapping in the height of the dwarf, intermediate-dwarf, and normal classes.

THE F_3 GENERATION

Seed from normal and from dwarf F_2 plants was selected at random for growing at Pendleton, Oreg., in the F_3 generation. A fairly large population was produced by the normal F_2 plants, but the dwarf progenies suffered from winter-killing.

The normal F_2 plants, according to the three-factor hypothesis, should produce in the F_3 generation a ratio of 19 true-breeding normal (tall) families to 6 segregating families. Two of the families should segregate in a ratio of 3 normal to 1 dwarf, and 4 families in a ratio of 13 normal to 3 dwarf plants. The dwarf F_2 plants should produce segregations in the F_3 generation of 7 true-breeding dwarf to 32 segregating families. Four types of segregation for dwarf and normal plants should occur among the 32 segregating families in the following ratios: 16, 3 : 1; 4, 13 : 3; 4, 9 : 7; and 8, 39 : 25.

The ratio of true-breeding to segregating families from both normal and dwarf F_2 plants was close to the calculated results (table 3). A study of the relative number of normal and dwarf plants in individual rows (families) from normal F_2 plants showed, however, a deficiency of dwarf plants in nearly all rows, based on the expected ratios 3 : 1 and 13 : 3. A number of these rows from each of the 4 crosses contained only 1 to 4 dwarfs among 50 or more plants. These may be normal progenies in which some chromosomal aberration has resulted in the production of dwarfs. Thompson (*11*) grew F_4 progenies from normal plants selected from four F_3 families, each of which likewise had given but few dwarfs. He found dwarfs in the progeny of but a single plant and concluded that such F_3 rows were in reality true-breeding normals.

TABLE 3.—*Behavior in the F_3 generation of the progenies of F_2 plants of four wheat crosses at Pendleton, Oreg., 1934*

Cross	Progenies of normal F₂ plants						Progenies of dwarf F₂ plants					
	Total	Observed		Calculated [1]		Deviation [2]	Total	Observed		Calculated [3]		Deviation [2]
		Normal	Segregating	Normal	Segregating			Dwarf	Segregating	Dwarf	Segregating	
	No.	*No.*	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*	*No.*	*No.*	
Jenkin × Turkey-Florence and reciprocal	50	40	10	38.0	12.0	2.0±2.04	36	3	33	6	30	3±1.55
Turkey Florence × Federation	61	44	17	46.4	14.6	2.4±2.25	29	1	28	5	24	4±1.39
Turkey-Florence × Baart	51	39	12	38.8	12.2	.2±2.06	33	2	31	6	27	4±1.49
Hussar-Hohenheimer × Jenkin and reciprocal	38	24	14	28.9	9.1	4.9±1.78	25	8	17	4	21	4±1.29
All crosses	200	147	53	152.0	48.0	5.0±4.07	123	14	109	22	101	8±2.87

[1] Based upon the ratio of 19 true-breeding normal to 6 segregating.
[2] Probable errors calculated by the formula 0.6745 $\sqrt{pqn}$.
[3] Based upon the ratio of 7 true-breeding dwarf to 32 segregating.

If the families having a low number of dwarfs are included with the true-breeding normal families, a very good fit for the 19 true-breeding to 6 segregating lines is obtained.

The number of true-breeding dwarf families was less than the expected 7:32 ratio in three of the crosses and above the expected number in one cross. Deviations from the calculated number were less than three times the probable error in all crosses. The somewhat small number of dwarf families possibly may be accounted for by a higher mortality in the F_2 among the homozygous than among the heterozygous dwarf plants.

When the distributions for the individual rows in the various crosses were examined for conformity to the expected ratios, it was evident that the families could not be separated accurately into the expected types of segregation. However, a number of the families from normal F_2 plants, particularly in Jenkin $\times$ Turkey-Florence and Turkey-Florence $\times$ Federation crosses, segregated in satisfactory 3:1 and 13:3 ratios. It is clear that the dwarf plants suffered considerable killing.

The F_3 segregations in the Turkey-Florence $\times$ Baart cross are shown by individual families in table 4.

TABLE 4.—*Number of normal and dwarf plants in F_3 families from F_2 normal plants in the Turkey-Florence $\times$ Baart cross at Pendleton, Oreg., 1934*

Plants from normal F_2 plants				Plants from dwarf F_2 plants			
Normal	Dwarf	Total	Breeding behavior	Dwarf	Normal	Total	Probable breeding group
Number	*Number*	*Number*		*Number*	*Number*	*Number*	
46	17	63	Segregating	13	4	17	3 *D*:1 *N*.
53	17	70	____do____	26	8	34	Do.
36	27	63	____do____	21	6	27	Do.
47	27	74	____do____	48	20	68	Do.
70	7	77	____do____	46	18	64	Do.
78	6	84	____do____	70	25	95	Do.
56	11	67	____do____	36	13	49	Do.
59	6	65	____do____	51	17	68	Do.
63	14	77	____do____	42	18	60	Do.
58	10	68	____do____	35	9	44	13 *D*:3 *N*.
69	6	75	____do____	30	23	53	9 *D*:7 *N*.
74	3	77	Normal with few dwarfs	39	48	87	Do.
68	2	70	____do____	40	43	83	Do.
57	2	59	____do____	29	38	67	Do.
78	1	79	____do____	47	32	79	Do.
80	1	81	____do____	15	20	35	Do.
2,966	0	2,966	All normal [1]	19	26	45	Do.
				34	32	66	Do.
				37	31	68	Do.
				8	15	23	Do.
				34	50	84	Do.
				19	32	51	Do.
				9	21	30	Do.
				16	33	49	Do.
				53	28	81	39 *D*:25 *N*.
				48	21	69	Do.
				43	27	70	Do.
				43	28	71	Do.
				45	20	65	Do.
				40	27	67	Do.
				35	16	51	Do.
				171 [2]	0	171	True-breeding dwarf.

[1] 35 rows. [2] 2 rows.

All segregating families grown from the dwarf F_2 plants had a fairly good proportion of dwarfs, but according to expectation there should have been a preponderance of dwarf plants in all of the families

that segregated. This was the case in the majority of the families only in the Turkey-Florence × Baart cross, data for which are shown in table 4. In this cross families were obtained that indicated the expected segregating dwarf to normal ratios of 3:1, 13:3, 9:7, and 39:25. The proportion of families segregating into 3:1 and 13:3 ratios was less than the calculated values, owing to shortages of dwarf plants.

Two families in the Hussar-Hohenheimer cross were all dwarf except for one normal plant in one family and two normal plants in the other. It is probable that these plants were the results of natural crossing by normal plants. Tingey (*12*) showed that a fairly high percentage of natural crossing on dwarf plants by pollen from normal plants may occur.

DISCUSSION

When a combination of parents gives rise to a new character expression such as dwarfness in the F_1 generation, it is evident that some sort of complementary-factor relationship is responsible. It has been shown by a number of investigators that dwarfing in wheat is due to a dominant factor *D* for dwarfing and that this factor is hypostatic to a factor *I* (or *N*) for normal height. A number of normal wheats have been shown to possess the dominant *IIDD* factors, and other normal varieties the recessive *iidd* factors. To explain the production of dwarfs in F_1 from normal varieties, Thompson (*11*) assumed a third factor (*E*) for the dwarf-factor complex, which inhibits or neutralizes the *I* factor.

This scheme satisfactorily explains the general situation where F_1 dwarfs are produced from normal parents. Contrary to Thompson's point of view, however, a modified complementary-factor scheme also may be used to explain the results obtained in the crosses described by the writers and by Thompson (*11*) without assuming an inhibitor-of-an-inhibitor relationship, if his *E* factor is assumed to be a second factor for dwarfing which is not capable of producing dwarfs alone, or in the absence of *I*, but which with *D* suppresses the expression of the *I* factor. The factors *D* and *E* would thus represent different capacities for dwarfing, and the factors *IIDD* would be contributed by one parent and the *EE* factor by the other parent when dwarfs are produced in the F_1 generation. This factor hypothesis would produce the same segregations as in Thompson's scheme.

The occurrence of occasional aberrant dwarf plants in apparently normal families has been elucidated by the cytological researches of both Goulden (*5*) and Thompson. Thompson (*11, p. 346*) states that—

In view of the difficulty in formulating a completely satisfactory genetic interpretation, one might be inclined to attribute dwarfishness to chromosome irregularity. Such a proceeding would not be justified, however, since the F_1, though dwarf, had the full complement of 21 chromosomes as did dwarfs of later generations. It seems clear that dwarfness is due to genetic factors.

He concludes that the appearance of occasional unexpected dwarf plants may very well be due to the chromosome irregularities observed.

The duplication of chromosome sets (or genoms), which is believed to have taken place in the evolution of wheat, makes it entirely possible that chromosome homology may exist to a greater or less degree among at least some of the chromosome pairs. As stated by Goulden

(*5*), "There may in some cases be sufficient affinity between chromosomes that are not strictly homologous to bring about different types of pairing." Irregular pairing would result in occasional factor combinations that would afford opportunity for expression of the dwarf. factor.

The results of the experiments reported in this paper indicate the genotypic composition for dwarfing in the wheat varieties used. The three-factor combination includes the normal (or dwarf-inhibiting) factor (*I*), a dominant factor (*D*), and a complementary dwarfing factor (*E*). The only parental combinations of factors which together would yield a dwarf F₁ progeny from normal parents would be *IIDDee* and *iiddEE*.

Turkey-Florence crossed with Jenkin, Federation, Baart, and the two Arco selections 8118 and 8120 produced all dwarfs in F₁, as did Hussar-Hohenheimer crossed with Jenkin, and the Arco selections. The genotypes of Turkey-Florence and Hussar-Hohenheimer must be identical, since crosses with the same varieties gave similar results. Also the five varieties with which these two were crossed must have the same genotype for the same reason. Tingey (*12*) showed in his studies, where two factors for dwarfing were involved, that the genotype of Federation was *IIDD*. The senior writer (*4*), in his studies of dwarfs in backcrosses, found that Jenkin contained the dominant factor *IIDD* and Quality the recessive factors *iidd*. Since Baart and the two Arco strains produced the same results as Jenkin and Federation, all must have had the dominant factors *IIDD*.

The third factor, *EE*, must have been contributed by Turkey-Florence and Hussar-Hohenheimer. The genotype for dwarfing of Jenkin, Federation, Baart, and the two Arco selections apparently is *IIDDee* and that of Turkey-Florence and Hussar-Hohenheimer *iiddEE*.

SUMMARY

Dwarfs were obtained in the F₁ generation in eight crosses between normal (tall) varieties of wheat at Moscow, Idaho, in 1931 and 1932. Turkey-Florence was used in five crosses, and Hussar-Hohenheimer in three crosses. In 1931 Turkey-Florence was crossed with Jenkin, Federation, and Baart, and Hussar-Hohenheimer was crossed with Jenkin. In 1932 both Turkey-Florence and Hussar-Hohenheimer were crossed with the two selections of Arco.

The height of F₁ dwarf plants averaged from 15 to 17 inches, and that of the normal parents from 33 to 44 inches. The average weight of kernel of F₁ dwarf plants was less than that of the parents.

The segregation of dwarf and normal plants was studied in the F₂ and F₃ generations in the four crosses made in 1931. Winter-killing caused considerable loss of F₂ plants. The heaviest losses seemingly occurred among the dwarfs.

The number of normal plants, based on a three-factor difference and a ratio of 39 dwarf to 25 normal plants, was close to the expected in one of the F₂ crosses.

The expected ratio of true-breeding to segregating families from both normal and dwarf F₂ plants was verified by the F₃ results. Satisfactory fits were obtained in all crosses. In general, dwarfs in individual families were too few in number to satisfy the expected ratios.

Dwarf F_1 plants from crosses between normal varieties indicate complementary factors. To explain the results on a complementary-factor basis, a second dwarfing factor, E, may be assumed, which, with the established D factor, is dominant over I. The E factor alone, unlike the dominant D, is not capable of producing dwarfs in the absence of I.

The genotype of the varieties Turkey-Florence and Hussar-Hohenheimer, accordingly, is *iiddEE*, and that of the other varieties used *IIDDee*.

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PEA MOSAIC AND ITS RELATION TO OTHER LEGUME MOSAIC VIRUSES[1]

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INTRODUCTION

Pea disease surveys made in a number of Western States revealed the fact that mosaic was usually more prevalent along the borders of fields than elsewhere. The results of these surveys suggested the possibility that the legumes growing along irrigation ditches, road-ways, and borders of fields might be the source of the viruses that cause the mosaic diseases of pea (*Pisum sativum* L.).

This paper embodies the results of a study of the common pea mosaic virus in comparison with viruses from red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), white sweetclover (*Melilotus alba* Desr.), and alsike clover (*T. hybridum* L.). The paper presents (1) a description of the symptoms of the common pea mosaic, together with the symptoms of other legume mosaic diseases on their own hosts and on the pea; (2) preliminary data on the susceptibility and resistance of 42 varieties of peas to all these viruses; (3) methods for differentiating the viruses; and (4) data on the transmission of the common pea mosaic virus.

It seems probable that other viruses may be present in the various legumes which may sometimes produce reactions different from those recorded here. Likewise, viruses from other mosaic-infected legumes not included in this paper may produce symptoms on peas different from or similar to those here reported. Detailed work on virus properties may reveal that in some cases a given virus heretofore treated as a unit is composed of two or more separable components.

REVIEW OF LITERATURE

The first report of the occurrence of sweetclover and red clover mosaic was made by Elliott (*4*),[3] who showed that the virus was infectious to *Medicago arabica* (L.) Huds. and *Vicia faba* L. Dickson (*2*) reported that pea mosaic occurred in Quebec and that he had obtained successful inoculations from the garden pea to the sweet pea (*Lathyrus odoratus* L.), but negative results when cross inoculations to pea and bean were made. He also presented data on seed transmission of pea mosaic. Taubenhaus (*11*) reported the occurrence of sweet pea mosaic in Delaware and described a series of experiments which indicated that it was transmitted both by artificial inoculation and by aphids.

Doolittle and Jones (*3*) reported a mosaic disease of garden pea which they transmitted to red clover and sweet pea. They were

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unsuccessful in transmitting the virus of sweetclover and bean to pea and sweet pea.

Böning (*1*) described a mosaic disease of broadbean (*Vicia faba*), the virus of which was infectious to pea, crimson clover (*Trifolium incarnatum* L.), and red clover. He considered it as possibly identical with the pea mosaic described by Doolittle and Jones (*3*).

Merkel (*5*) believed that the same virus was responsible for the mosaic symptoms of the following hosts: *Phaseolus vulgaris*, *Pisum sativum*, *Lathyrus odoratus*, *Lupinus luteus* L., *Melilotus altissima* Thu., *Trifolium pratense*, *T. hybridum*, *T. repens*, *Anthyllis vulneraria* L., and *Vicia faba*.

Zaumeyer (*13*) and Zaumeyer and Wade (*14*, *15*) reported the transmission of a pea mosaic to bean (*Phaseolus vulgaris*). They also reported that a number of different legume mosaic viruses, including those of red clover, white clover, and white sweetclover, were transmissible to bean, pea, and sweet pea. Osborn (*7*, *8*) working on the transmission of two distinct viruses of pea, reported that one of them required an incubation period of about 18 hours in the aphids *Macrosiphum gei* Koch and *M. pisi* [*Illinoia pisi*] Kalt., while the other was transmissible within 30 minutes after it was picked up. Snyder (*10*) described a pea mosaic in California which produced serious pod deformation and which he believed was caused by a different virus from the common pea mosaic virus.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Pea mosaic has been found wherever peas are grown in the United States, and it is likely that the several legume viruses which are infectious to pea have equally as wide a distribution. Heretofore these diseases have not been differentiated, and since the symptoms produced by the various legume viruses on the pea are somewhat similar, it is not unlikely that some of the mosaic reported on pea was actually not the common pea mosaic. The virus diseases discussed in this paper apparently do not cause as much damage to pea varieties used for canning as they do to market-garden varieties. In sections where the latter are produced either for market or for seed the mosaic diseases are frequently epidemic, often causing considerable reduction in yield. Differences in varietal susceptibility possibly account in part for the greater damage to market-garden varieties.

Pea mosaic was first reported by Dickson (*2*) from Quebec, Canada, in 1922. Martin and Haensler [4] found it in New Jersey in 1924. Doolittle and Jones (*3*) found it in many commercial fields in Wisconsin in 1924, reporting that as many as 15 percent of the plants were infected. They also stated that Brotherton had reported it orally as occurring in considerable amount in Michigan in 1924. In 1928 Linford [5] reported it from nine States, from Utah to the Atlantic coast. It reached its maximum severity in New Jersey and Maryland. Snyder (*10*) described a pea mosaic in California in 1934 which caused considerable damage to the pods. The writers found it of considerable importance in certain sections of Colorado in 1932 and

[4] MARTIN, W. H., and HAENSLER, C. M. PEA DISEASES IN NEW JERSEY. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 8: 43–53. 1924. [Mimeographed.]
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1933, especially in peas grown at the higher altitudes. It was of less importance there in 1934 and 1935. Mosaic was also found in 1934 in Wyoming and southern Idaho and was reported to be common in sections of northern Idaho and Washington. It was observed more extensively in the market-garden varieties than in the canning and field varieties. Reports from the market-garden sections of the States of Washington, Oregon, and California indicate that it is of major importance there.

It appears that the disease, although not a serious factor in the curtailment of production except in certain localities, has become more widespread during the past few years and may be of some concern in the future. With the increased use of yellow and white sweetclovers as green-manure crops in certain of the western pea-seed-producing sections, it is possible that the mosaic of peas may increase because of the transmissibility of sweetclover mosaic to them.

Because of the similarity of symptoms of the common pea mosaic and the mosaics of related legumes on pea, little is known about the distribution of these various viruses. Experimental evidence has demonstrated that the common pea mosaic virus is carried in the seed in only a small amount, which indicates that seed transmission is usually not an important factor. Whether the viruses of other legumes inoculated to pea are carried in the seed in larger amounts is unknown.

Since aphids (*3, 5, 7, 8, 14, 15*) have been shown to transmit the common pea mosaic virus, and since other closely related viruses are transmissible to pea, it is not unlikely that much of the infection found in a given locality is correlated with the aphid population and the number of infected legumes in the vicinity of the pea-producing areas.

MATERIALS

SOURCE OF SEED

The seed of the pea varieties used in the experimental work was either furnished by American seed firms or grown by the writers. Pea seed from mosaic-infected plants was obtained from three different seed firms.

SOURCE OF VIRUSES

PEA MOSAIC

The common pea mosaic was collected from Dwarf Telephone pea plants grown in the southeastern part of Colorado, near Stonewall. Other material was secured from mosaic-infected Green Giant peas grown in Montana in 1933. The viruses from these two sources were identical. The experimental data presented here are based on the virus from Colorado. The virus extract from infected plants produces typical systemic mosaic symptoms (fig. 1, *A–D, H, I, K, L*), which are described later.

RED CLOVER MOSAIC

The red clover virus used in these studies came from mosaic-infected plants collected near Rosslyn, Va. This virus is believed to be identical with the one described by Doolittle and Jones (*3*) as being infectious to pea and sweet pea. It produces only mottled symptoms when inoculated to pea.
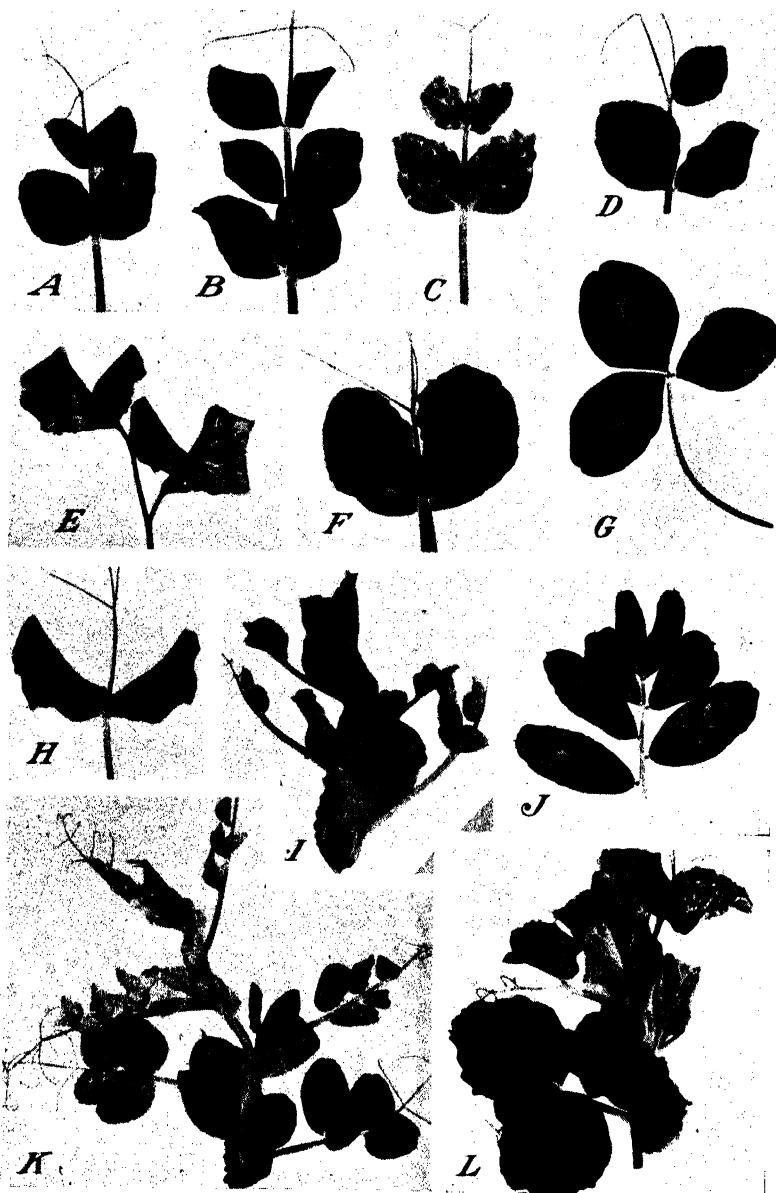
FIGURE 1.—Variations in leaf symptoms of pea mosaic on several pea varieties and of red clover mosaic on red clover, sweet pea, and *Vicia faba: A, B, C, D, K,* variations in symptoms on Green Giant peas from mild mottle to vein clearing and extreme chlorosis; *E, F,* mottling on flower and leaf of Baltimore Rose sweet pea; *G,* mottle on red clover; *H, I, L,* pea mosaic on Dwarf Telephone peas, showing inward curling, cupping, and delayed opening of leaves and stipules and heavy mottle and twisting of leaflets; *J,* leaves of *Vicia faba* showing mottling.

WHITE CLOVER MOSAIC

The virus of white clover mosaic was secured from material collected near Rosslyn, Va., and in the District of Columbia. The symptoms produced by both of these viruses were identical, both mottled and local necrotic symptoms appearing on many pea varieties (fig. 2, *D*, *E*, *F*).

WHITE SWEETCLOVER MOSAIC

Collections of white sweetclover mosaic virus were made from white sweetclover plants grown near Rosslyn, Va., and in the District of Columbia. Like the white clover virus, it produces both mottled and necrotic symptoms (fig. 3, *A*, *D*, *E*, *F*, *G*, *J*, *K*). The mottled symptoms, however, were dissimilar to those produced by the white clover virus, while the necrotic lesions appeared identical (fig. 3, *K*).

ALSIKE CLOVER MOSAIC

Specimens containing the virus of alsike clover mosaic were collected from alsike clover plants growing in northeastern Colorado. This virus produces only the mottled symptoms on peas, which are unlike those of the other viruses herein described (fig. 4). Other viruses from alsike clover, not discussed herein, show specific differences from this virus. For the sake of clarity, the virus reported herein will be designated as alsike clover virus 1.

METHODS

Most of the experimental work was carried on in the greenhouse, where the temperature varied from 18° to 25° C. The work on seed transmission was conducted both in the greenhouse at Rosslyn, Va., and in field plots in Colorado. In order that the different viruses might be readily available at all times they were transferred to *Vicia faba* plants, which served as a convenient host for culturing them.

The inoculum was prepared by grinding up mosaic-infected plants in a mortar. Plants were inoculated by briskly rubbing four to six leaflets with a cheesecloth pad saturated in the inoculum. They were not inoculated until they had grown to the eighth- to tenth-node stage. With this technique high percentages of infection were secured only with the white clover and white sweetclover mosaic viruses. The viruses of pea mosaic, red clover mosaic, and alsike clover mosaic were not readily transferred to peas.

Hoping to secure higher percentages of infection, especially for the studies of varietal resistance and susceptibility, the writers used the pea aphid (*Illinoia pisi* (Kalt.)) to transmit the red clover mosaic virus. The aphids were allowed to feed on *Vicia faba* infected with the red clover virus and later were transferred to healthy peas under cages. This method likewise failed to produce satisfactory infection.

Osborn (*8*) reports similar difficulties in securing transmission of pea mosaic by both the rubbing technique and the needle-prick method. Later studies, which are not reported in this paper, have shown that with the use of carborundum powder as an abrasive, higher percentages of infection may be obtained.
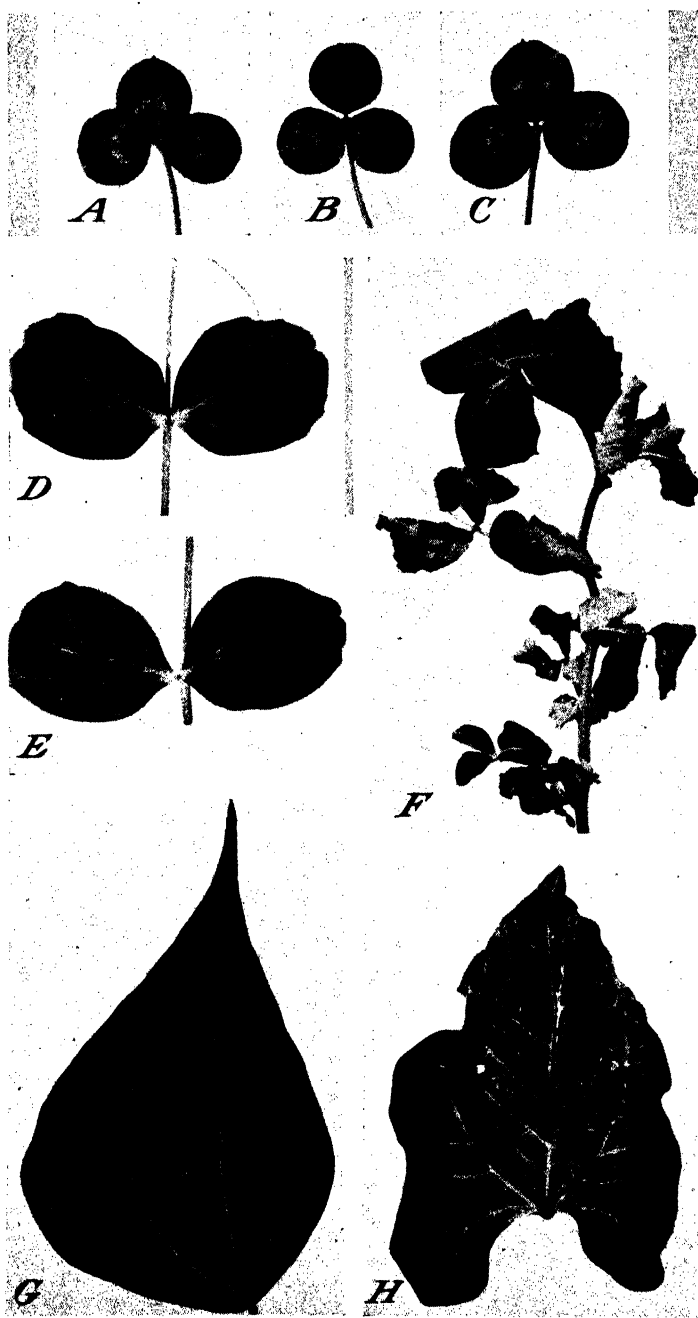
FIGURE 2.—Symptoms of white clover mosaic on white clover, peas, and beans: *A, B, C,* variations in mottle on white clover; *D, E,* mild mottle symptoms on Alderman pea; *F,* necrotic symptoms on Green Giant pea; *G, H,* systemic and local lesions on Stringless Green Refugee bean.

FIGURE 3.—Symptoms of white sweetclover mosaic on white sweetclover, peas, and beans: *A, D, F, J,* variations in mottle and chlorosis on Green Giant pea; *B, C,* variations in mottle on white sweetclover; *E,* mottle on Tall Telephone pea; *G,* mottle, delayed opening, and curling of leaflets on Bruce pea; *H, I,* downward curling of leaflets at the pulvini and mottle on Stringless Green Refugee bean; *K,* necrotic symptoms on Green Giant pea.

SYMPTOMS

The descriptions of the symptoms produced by the various viruses on the pea are based on the symptoms as they appear on the Dwarf Telephone and Green Giant varieties artificially inoculated in the
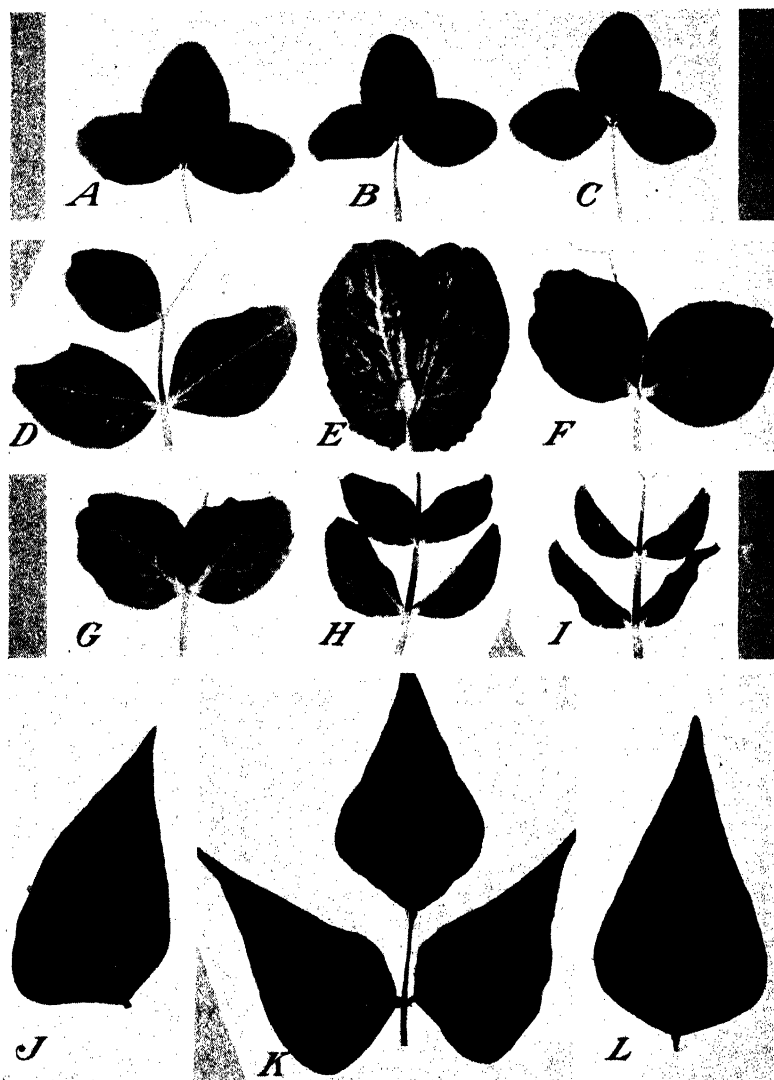
FIGURE 4.—Symptoms produced by the alsike clover mosaic virus on various hosts: *A, B, C,* symptoms on alsike clover; *D, E, F, G,* variations in mottle symptoms on leaves and stipules of Horsford pea; *H, I,* symptoms on leaflets of Tall Telephone pea; *J, L,* healthy leaves of Stringless Green Refugee bean; *K,* symptoms on Stringless Green Refugee bean.

greenhouse. In the case of alsike clover mosaic virus 1, the symptoms are described as they appear on the Tall Telephone variety. Although the symptoms produced by several of these viruses differ in certain

respects from one another, still there are certain overlapping similarities which make it difficult to differentiate clearly all of the viruses by symptomatology alone.

COMMON PEA MOSAIC

The first noticeable symptom produced by the virus of the common pea mosaic appears on the leaves and stipules (fig. 1, *A–D*) above the point of infection as a very slight chlorosis, the leaves being lighter green than normal. Very striking characteristics of young infected plants are the failure of the leaves to unfold as quickly as the leaves of an uninfected plant and the slightly wavy leaf edges (fig. 1, *H, I*). Later a mottling appears, due to the presence of small indistinct yellow spots located between the veins. The yellow areas increase in size, and dark-green irregularly shaped areas appear between the larger veins, producing an intense mottle. A pronounced vein clearing often appears (fig. 1, *K*), which increases in intensity from the lower to the upper branches. The tissue between the veins may vary from a moderately dark to a very light green to yellow. Infrequently, instead of a vein clearing, the green tissue persists in the region immediately adjacent to the veins and veinlets (fig. 1, *D*), while the area between the veins may vary from a light green to a yellow.

In the case of plants from infected seed, the symptoms are very pronounced. In certain cases an entire leaflet may be devoid of chlorophyll, with only a small amount of green pigment present at the base of the petiole (fig. 1, *K*). In still other instances all the leaves and stipules of a branch may be yellow, such leaves always being smaller than normal (fig. 1, *K*). Where a leaflet is only partially etiolated it may be decidedly malformed, wrinkled, puckered, or blistered, with the edges curled upward (fig. 1, *I, L*). The uppermost leaves of a severely infected plant do not open as readily as those of a normal plant, and often they remain folded tightly together. The symptoms are always manifested more clearly in the uppermost part of the plant (fig. 1, *L*).

When the infection occurs early in the life of the plant a stunting may result, but when it occurs later little reduction in size of the plant is noted. The pods of an infected plant may be somewhat malformed and distorted and often reduced in size.

RED CLOVER MOSAIC

The symptoms of the red clover mosaic virus first appear on the young center leaves of red clover as a mild mottle (fig. 1, *G*). Later the leaves become slightly streaked with yellow, which may sometimes extend over considerable portions of the leaves, with small islands or larger sectors of green tissue remaining. The islands of green, which are usually linear in shape, are frequently located adjacent to the veins. These green areas may follow a single vein or they may extend over three or four veins. Occasionally, in the case of severe infection, the leaves may show an arching with the midvein depressed. Stunting of infected plants is not common, and under conditions of high temperature the symptoms are readily masked.

RED CLOVER MOSAIC ON PEA

The symptoms produced by the virus of red clover mosaic when inoculated to pea are indistinguishable from those produced by the common pea mosaic virus.

WHITE CLOVER MOSAIC

The initial symptom of white clover mosaic is a very mild stipple-like mottle of the leaflet due to the presence of small light-green areas between the veins (fig. 2, *A, B, C*). Later the leaflet shows a more distinct mottle of light- and dark-green areas. The light-green areas enlarge and take on a light yellowish color, which may produce a considerable streaking of the leaves. The streaks sometimes follow the veins and may extend from the midrib to the margin of the leaflet. In other cases a considerable portion of the leaflet may be chlorotic, the remaining green areas usually being found adjacent to the midrib and extending outward toward the laterals. Islands of dark-green tissue frequently occur in other portions of the leaflet, either along the periphery or particularly at the base. Infected leaves are usually not distorted, but in some cases they are smaller than the normal leaves. Environmental conditions such as low or extremely high temperatures readily mask the symptoms.

WHITE CLOVER MOSAIC ON PEA

The white clover mosaic virus when inoculated to pea produces necrotic lesions and mild mottling. The necrotic lesions appear in about 7 to 10 days after inoculation. The first symptoms are a drooping of the inoculated leaves (fig. 2, *F*), a slight yellowing of the entire plant, and the eventual death of the inoculated leaves. The midvein on the upper side of the stipule nearest the point of inoculation takes on a purplish hue, and most of the veins on the lower side of the leaflets become discolored. The stipules recurve and the leaflets stiffen and curve downward. The uppermost leaves are subnormal in size, do not unfold as readily as normal leaves, and take on a crinkled appearance. The stems shrivel and turn to an indistinct purple, while the lower leaves shrivel and die. The vascular bundles of infected stems become discolored and necrotic; fungi may be isolated from them occasionally, but in no case have they proved pathogenic. It is likely that infected plants become weakened by the virus and that saprophytic fungi enter the root system. Infected plants often die, but it is believed that death does not result directly from the virus but from the virus in conjunction with organisms found in the roots and stems.

Plants infected with the white clover virus are not stunted, and the symptoms may be readily overlooked. The mottled symptoms are first manifested by small light-green areas appearing between the veins (fig. 2, *D, E*). They are not readily observed except by transmitted light. Later these leaflets become slightly chlorotic and blotches of light green appear, which are not bounded by the veins but extend throughout the entire leaflet. The infected leaflets are normal in size and are not malformed. Sometimes chlorosis may occur only along the edges of the leaves, the normal green remaining along the midvein and part way along the branch veins. More frequently the symptoms are noticeable only toward the base of the plant, the upper leaves appearing normal.

WHITE SWEETCLOVER MOSAIC

The first symptoms of white sweetclover mosaic appear as small, somewhat indistinct light-yellow spots on the leaves. These spots enlarge, coalesce, and may finally cover almost the entire leaflet, producing a speckled effect (fig. 3, *B*, *C*). The infected leaflet may become quite chlorotic with only small areas of green remaining, usually adjacent to the midvein and to some of the laterals. In other cases there may be a clearing of the veins with darker-green areas between them. Frequently the portion of the leaflet immediately adjacent to the midrib and extending some distance from it may be cleared, leaving only portions of the leaf margin a darker green. Under greenhouse conditions, especially in cases of severe infection, the leaves may be dwarfed and ruffled. In the field the infected plant may be stunted early in the spring, but later in the season it may be almost normal in size and the symptoms may be masked, especially if temperatures are high.

WHITE SWEETCLOVER MOSAIC ON PEA

The white sweetclover mosaic virus is similar to the white clover mosaic virus in that it produces both the necrotic and systemic symptoms on pea. The necrotic lesions are essentially identical with those produced by the white clover virus and it is impossible to differentiate them. The mottled symptoms, however, are decidedly different, and it is in this respect that the two viruses can be separated. The virus makes its first appearance as small, indistinct light-green spots located between the veins. These later enlarge and become more distinct and the entire leaflet may be mottled (fig. 3, *E*, *F*, *G*). Finally a light green replaces the dark green in the tissue except the dark green adjacent to the veins (fig. 3, *E*), which may persist. The light-green areas finally become yellow, producing a marblelike effect. The lower leaves may appear entirely normal at a distance of four nodes from the point of inoculation. The uppermost leaves, on the other hand, are smaller, sometimes greatly reduced in size, decidedly puckered, blistered, and twisted, often with an upward curl of the leaf periphery (fig. 3, *G*). Some of the leaves may have large blotches of yellow interspersed with areas of green (fig. 3, *A*, *D*, *J*). The yellow tissue apparently fails to develop normally, and with the continued growth of the cells in the normal green tissue the infected leaflets become distorted and malformed. Occasionally one stipule may be almost normal, whereas the opposite one may be greatly distorted and remain decidedly reduced in size. In severe cases of infection, the plant may be dwarfed, and in such instances the pods fail to develop normally.

In certain respects the mosaic symptoms produced on pea by the white sweetclover virus are somewhat similar to those of pea mosaic. However, the production of large yellow blotches in the youngest leaves, accompanied by a decided malformation of these leaves, provides a means whereby the white sweetclover virus can be differentiated from the pea mosaic virus. In mild cases of infection the symptoms may be similar to those produced by the white clover virus.

ALSIKE CLOVER MOSAIC

The symptoms manifested by the alsike clover virus 1 (fig. 4, *A*, *B*, *C*) are essentially the same as those of white clover mosaic.

ALSIKE CLOVER MOSAIC VIRUS 1 ON PEA

Alsike clover mosaic virus 1 produces only the mottled symptoms on pea. In this respect it differs from the white clover virus, which it resembles, and from the white sweetclover virus. The symptoms are first noted as a mild stippling of the leaves (fig. 4, *D–G*), produced by small light-green areas interspersed among the normal green of the leaflet. Only a slight chlorosis is produced, and frequently the darker green tissue is more noticeable along the veins and veinlets (fig. 4, *F*, *G*). The infected leaves and stipules are normal in size and are not malformed. These characteristic features differentiate it from the pea and red clover viruses, which produce marked symptoms on Green Giant and Dwarf Telephone varieties, as described previously (fig. 1).

On other varieties, such as Tall Telephone, the alsike clover mosaic virus 1 produces very conspicuous symptoms, such as a decided chlorosis of the leaves and stipules with only small areas of light-green tissue remaining. These areas are usually found at the base of the leaflet and frequently adjacent to the vein and veinlets. In other instances these darker areas are interveinal and appear as blotches or small islands of green. Infected pea plants are not stunted as they are when infected with the white sweetclover virus. The infected leaflets do not unfold normally but are cupped upward (fig. 4, *H*, *I*), especially toward the tip of the leaflet. Such leaflets are smaller than normal, but are not otherwise malformed.

PEA VARIETAL RESISTANCE AND SUSCEPTIBILITY

The several viruses infectious to pea are not readily transmitted by the usual mechanical methods. Because of this the results here reported are not entirely conclusive. However, they do indicate that certain varieties are decidedly more resistant than others.

Forty-two varieties of peas were inoculated with the several viruses under greenhouse conditions. Although it is believed that the red clover mosaic virus is similar to if not identical with the pea mosaic, it was included in these studies. The plants used in this test were grown in greenhouse benches and inoculated with an undiluted extract from the various mosaics according to the method already described. In addition to inoculation by this method, the red clover virus was also transmitted to peas by means of the pea aphid. The viruses used in the mechanical inoculations were either extracted from mosaic tissues of the original host plant or from peas or *Vicia faba* infected with the various mosaic viruses. The results in all cases were comparable. All of the varieties tested were not inoculated at one time, but were run in several series. As may be seen from table 1, the varieties were divided into three general groups, according to use and season.

TABLE 1.—*Susceptibility and resistance of different varieties of pea to mosaic viruses of pea, red clover, white clover, white sweetclover, and alsike clover*

Group and variety	Pea mosaic, mottled		Red clover mosaic, mottled				White clover mosaic				White sweetclover mosaic				Alsike clover mosaic 1, mottled	
			Mechanical transmission		Aphid transmission		Mottled		Necrotic		Mottled		Necrotic			
	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Canning:																
Early:																
Alaska	11	6a	20	1c	11	0	22	3b, 6c	25	18a, 3b, 2c	32	6a, 11b	10	6a, 4c	31	7a, 4b
Surprise	10	0	11	0	10	2c	29	0	29	3b, 16c	29	0	29	3b, 6c	10	0
Wisconsin Early Sweet	10	0	10	0	12	0	10	0	6	5b, 1d	10	0	10	10c	10	0
Midseason:																
Bruce	9	1a	12	0	10	4a, 4b	10	10c	10	6c	10	3a	10	10c	11	3b
Green Admiral	10	7b	9	0	10	7b	24	23c	26	1b, 22c	12	5b, 4i	12	1b, 5c	9	3b
Green Giant	28	4a	15	0	12	3a	21	7b, 12a	46	3a, 31b, 5c, 7d	20	1.5c	43	13b, 13c, 17d	23	100, 2a
Horsford	9	1a	10	0	10	8a	10	10b	10	5c	10	6c	10	0	9	8c
Perfection	10	0	9	1c	9	0	27	9b, 12c	27	1a, 1b, 23c	20	8b, 12c	20	2a, 7b, 7c	10	1c
Perfection (wilt resistant)	9	3c	10	0	11	0	25	21c	20	8a, 10b, 2c	20	7b, 12c	20	1a, 18b, 1c	8	8c
Prince of Wales	10	3b	8	0	12	3c	10	10c	10	10b	10	1a, 9c	10	10b	11	1a, 2b
Senator	10	3b	10	0	10	4a, 5b	10	8c	10	10b	10	6c	10	10b	10	0
Yellow Admiral	10	2b	9	0	10	3a, 3b	10	9c	10	10b	10	6c	10	10c	10	8c
Market garden:																
Early:																
Bliss Everbearing	11	7a	10	0	11	2a, 2c	16	14c	15	10c	17	3a	18	7c	8	1b
Extra Early	8	5c	12	4c	12	5c	24	12c	24	14a, 10b	17	8b, 9c	16	7a, 9c	11	1c
Gradus	10	2a	12	2c	10	10c	12	6a, 4c	12	2b, 10c	20	6b, 7c	32	9a, 1b, 5c	10	5a
Hundredfold	10	0	9	0	10	0	18	9b, 4c	18	9a, 10c	10	2a, 2b	8	8a	9	0
Laxtonian	10	3c	7	0	14	0	27	10c	34	9a, 10b	24	3b, 17c	31	10b, 11c, 7d	13	1b, 2c
Laxton Progress	12	1a	10	4b	8	6n	16	10c	17	22b, 5c, 7d	24	4c	24	9a, 11b, 1c	10	20
Laxton Superb	8	6c	11	10c	10	0	16	8c	20	8a, 8b, 1c	24	3b, 17c	26	4a, 14b, 3c	10	4c
Little Marvel	10	0	10	0	12	0	20	9c	11	5b, 8c	24	2b, 17c	19	9c	10	4c
Nott Excelsior	10	0	10	1c	10	0	10	6c	10	9c	19	1c	19		17	0
Pioneer	8	4b			9	60										

[1] All plants were inoculated by mechanical transmission except those inoculated with red clover mosaic virus, in which case transmission was made both mechanically and by means of aphids.

[2] *a* represents severe symptoms; *b*, moderate symptoms; *c*, mild symptoms; and *d*, death of plant.

TABLE 1.—*Susceptibility and resistance of different varieties of pea to mosaic viruses of pea, red clover, white clover, white sweetclover, and alsike clover*—Continued

Results of inoculation with virus indicated

Group and variety	Pea mosaic, mottled — Plants inoculated	Pea mosaic, mottled — Plants infected	Red clover mosaic, mottled; Mechanical transmission — Plants inoculated	Red clover mosaic, mottled; Mechanical transmission — Plants infected	Red clover mosaic, mottled; Aphid transmission — Plants inoculated	Red clover mosaic, mottled; Aphid transmission — Plants infected	White clover mosaic; Mottled — Plants inoculated	White clover mosaic; Mottled — Plants infected	White clover mosaic; Necrotic — Plants inoculated	White clover mosaic; Necrotic — Plants infected	White sweetclover mosaic; Mottled — Plants inoculated	White sweetclover mosaic; Mottled — Plants infected	White sweetclover mosaic; Necrotic — Plants inoculated	White sweetclover mosaic; Necrotic — Plants infected	Alsike clover mosaic 1, mottled — Plants inoculated	Alsike clover mosaic 1, mottled — Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Market garden—Continued.																
Early—Continued.																
Premium Gem	10	0	10	0	9	0	10	10b	10	7a, 3d	9	8c	10	5a, 4c, 1d	10	0
Pride of the Market	10	6a	10	0	10	5b	13	8c	13	5b, 8c	10	6a, 4c	10	9c	9	2a, 3b, 2c
Thomas Laxton	10	0	12	3c	12	1b, 2c	33	2c	45	9a, 32b, 4c	19	3a, 1c	35	11a, 14b, 10c	21	8b
World Record	10	1a	10	4c	10	5a	34	26c	34	28a, 5b	21	1a, 12c	22	8a, 12b, 1c	10	3a
Midseason:																
Alderman	10	7a	11	3c	10	5a	32	22a	32	17a, 15b	18	1c	26	16a, 10b	11	5b
Champion of England	10	4a	11	6b	9	3a, 3b	10	4c	7	5b, 1c	6	2b, 1c	6	5b, 1c	10	8c
Dwarf Alderman	10	2a		2c	10	0	8	0	21	12b, 9c	10	10c	15	7b, 7c	10	8b
Dwarf Quite Content	18	5a			12	5a	10	0	10	5a, 5d	10	2a, 3c	9	2a, 3c, 3d	9	4b, 3c
Dwarf Telephone	26	13a, 6b	10	2b	10	1a, 2a	19	8b, 11c	38	32b, 4c	16	2a, 14b	33	14b, 17c	29	13b
Giant Stride	10	6a	10	2c	10	5a	10	4c	15	5a, 4c	13	1c	13	3c	18	3c
Lincoln	18	3a	10	0	15	11a	15	4c	15	2a	15	1c	16	4b, 12c	17	2b, 5c
Onward	18	3c			12	0	10	10b	25	10b, 5c	9	5b, 5c	9	7c	9	0
Phenomenon	8	2a			11	2b	10	9b	10	2b, 8c	9	9c	27	4a, 17b, 4c	10	1a
Profusion	10	0	7	0	10	8c	10	10c	10	10c	8	6a	8	8c	16	1b, 1c
Stratagem	10	6a			10	8a	12	10b	30	12b, 13c, 5d	10	1a, 6c	25	10b, 13c	13	4b
Tall Telephone	10	1a, 6b			11	6b	20	20c	39	18a, 21b	10	4b, 6c	15	7b, 7c	10	8b
Field:																
Austrian Winter	11	1c	10	3c	16	3c	11	1c	11	11c	11	1c	11	1c	10	2a
Black Eye Marrowfat	12	3b	11	2c	10	4b	10	3c	10	10c	10	1a, 3b	10	7b, 3c	12	5b
Maple	10	4a	12	0	11	3c	10	10c	10	6c	10	2a, 3c	10	10c		
White Eye Marrowfat	10	0	11	1b	10	0	10	6c	10	9c	10	1a, 3b, 2c	10	8b, 2c	10	9c

It is believed that varieties showing 50 percent or more of mosaic when inoculated artificially would be considered quite susceptible under field conditions, while those showing less would be considered moderately susceptible. In certain instances only a small percentage of mosaic-infected plants may be noted, but the symptoms may be very severe. In other instances a large percentage of the plants inoculated may show only mild symptoms.

PEA MOSAIC

Table 1 shows that of the 12 varieties inoculated in the canning group, 3 were not infected, 1 showed mild, 4 moderate, and 4 severe symptoms. Of those showing no infection, Surprise and Wisconsin Early Sweet are early varieties and Perfection is a midseason variety. Alaska, Bruce, Green Giant, and Horsford showed marked symptoms, but only in the Alaska were more than 50 percent of the plants infected.

In the 26 market-garden varieties, 6 were not infected, 1 showed moderate, 4 mild, and 13 severe symptoms, and 2 varieties showed both severe and moderate symptoms. Of the varieties that manifested severe symptoms, the following six showed 50 percent or more of infection: Bliss Everbearing, Pride of the Market, Alderman, Giant Stride, Dwarf Telephone, and Stratagem. In the field group the White Eye Marrowfat showed no infection while Maple exhibited severe symptoms. The Austrian winter and Black Eye Marrowfat showed mild and moderate symptoms, respectively.

RED CLOVER MOSAIC

In general the pea varietal reaction to the red clover mosaic virus was somewhat similar to the reaction to pea mosaic, which tends to substantiate the belief that these two viruses are similar. The results of the mechanical transmission are not comparable in every case with aphid transmission, since more infection resulted from the latter method than from the former. In the canning group, two varieties were not infected by either method, four showed mild symptoms, one moderate, and two severe. In three varieties both severe and moderate symptoms were noted. In the market-garden group six varieties were not infected by either method, four manifested mild symptoms, three only moderate symptoms, and two only severe symptoms, while two varieties showed both mild and moderate symptoms, three both moderate and severe, and six both mild and severe symptoms. In the varieties classified as field peas no resistance was noted. All varieties showed mild or moderate symptoms.

There appears to be some correlation between the susceptibility and resistance of the various varieties to the red clover and pea mosaic viruses. With the exception of Surprise, Perfection, Profusion, Thomas Laxton, and White Eye Marrowfat, all of the ten varieties showing no infection with the pea mosaic virus likewise were not infected with the red clover virus. These five varieties were only slightly susceptible to the red clover virus, and the symptoms produced were in general very mild (table 1). The wilt-resistant Perfection, Laxtonian, and Onward varieties, were mildly infected by the pea mosaic virus, but were not infected by the red clover virus. Since these minor variations in susceptibility and resistance are not

outstanding, they do not appear to indicate specific differences in the two viruses, but are probably due to a combination of factors, such as age of inoculated plants, differences in concentration of the virus used, or slight variations in inoculation methods. Practically all the varieties manifesting marked symptoms when infected with one virus also produced similar symptoms when infected with the other. The Alaska variety, however, showed only a small percentage of infection when inoculated with the red clover virus, but was quite susceptible to the pea mosaic virus.

WHITE CLOVER MOSAIC

Of 40 pea varieties inoculated with the white clover mosaic virus only 3, Wisconsin Early Sweet, Dwarf Quite Content, and Dwarf Alderman, showed no mottled symptoms. Very mild symptoms were produced in almost all cases, and the plants were not stunted. All of the other varieties except Alaska, Surprise, Little Marvel, Laxton Progress, Thomas Laxton, Giant Stride, Champion of England, Lincoln, and Black Eye Marrowfat were very susceptible. The only correlation between resistance to this virus and to the mosaic viruses of pea and red clover is found in the Wisconsin Early Sweet, which is resistant to the three viruses.

In addition to the mottled mosaic symptoms, the white clover virus also produced marked necrotic lesions on the stems and inoculated leaves. All of the varieties tested exhibited these symptoms, and in some cases the virus caused death of the plant. Giant Stride was the only variety that showed some tolerance. Although showing a high percentage of infected plants, all of the field varieties manifested only mild symptoms.

WHITE SWEETCLOVER MOSAIC

The white sweetclover mosaic virus, similar to the virus of white clover, produced both the mottled and necrotic symptoms when inoculated to peas. The mottled symptoms were noted on 38 varieties, while 2, Surprise and Wisconsin Early Sweet, both belonging to the canning group, were not infected. Three varieties, Laxton Progress, Nott Excelsior, and Giant Stride, showed a high degree of tolerance to the mottle symptom, 30 varieties were very susceptible, and 5 exhibited severe symptoms but showed less than 50-percent infection. Severe symptoms alone were noted in three varieties, while eight showed both severe and mild symptoms or severe and moderate symptoms. All other varieties manifested mild symptoms.

The Horsford was the only variety that did not develop necrotic lesions. All others except Bliss Everbearing, Nott Excelsior, Giant Stride, and Austrian Winter showed a high percentage of infection. Green Giant and Laxton Progress each showed an exceedingly high percentage of infection, ranging from mild symptoms to death of plants. One variety produced only severe symptoms, and in 14 varieties the symptoms varied from severe to less marked. In 11 varieties only mild symptoms were noted.

ALSIKE CLOVER MOSAIC 1

The varietal reaction of peas to the alsike clover mosaic virus 1 was somewhat similar to that of peas to the mosaic viruses of pea and

red clover. In the canning group three varieties, Surprise, Wisconsin Early Sweet, and Senator were not infected. In the market-garden group four varieties manifested no infection, while in the field group no resistance was shown by any variety. Of the seven noninfected varieties, four were not infected by the mosaic viruses of pea and red clover, one was not infected by the white clover mosaic virus which causes the mottled symptoms, and two were not infected by the virus of white sweetclover mosaic. Of the 33 susceptible varieties only 11 showed 50 percent or more infection. Severe symptoms alone were observed in four varieties, while in three both severe and milder symptoms were noted; of these seven varieties, five were in the class showing a low percentage of infection.

SUMMARY OF VARIETAL TESTS

The data presented in table 1 show that the viruses of white clover and white sweetclover are more infectious to pea than are the other viruses. The data also show that the highest percentage of noninfected varieties belong to the canning group. Of the 12 varieties in this group, the following number of varieties showed no infection: 3 when inoculated with pea mosaic virus, 2 with red clover virus, 1 with the white clover virus which produces mottled symptoms, 2 with the white sweetclover virus which produces mottled symptoms, and 3 with alsike clover virus 1. Of the 26 market-garden varieties, the following number of varieties were not infected: 6 by the pea virus, 6 by the red clover virus, 2 by the white clover virus which produces mottled symptoms, and 4 by the alsike clover virus 1. In the field group, comprising four varieties, one, the White Eye Marrowfat, was not infected by the pea mosaic virus. The viruses of the other mosaics were infectious to all the varieties of this group.

DIFFERENTIATION OF THE VIRUSES

Although, in general, specific differences in symptoms are manifested when certain pea varieties are inoculated with the various legume viruses, there are some overlapping features which make it difficult at times to differentiate them clearly. Varietal variations as well as environmental conditions may alter the symptoms and make symptomatological separation of the viruses extremely difficult.

In an earlier paper by the writers (*15*) extensive tests were reported on the host range of the mosaic viruses of white clover, white sweetclover, red clover, and of a pea mosaic virus designated as pea mosaic 2, which was distinct from the virus of the common pea mosaic. Host-range studies were not attempted with the viruses of the common pea mosaic and the alsike clover mosaic. It seemed likely that the virus of the common pea mosaic would cause reactions similar to those produced by the red clover mosaic virus, because of the identity of symptoms and reactions of these two viruses on pea varieties. It was also believed that the effects of the alsike clover mosaic virus 1 would be similar to those of pea mosaic virus 2, which it somewhat resembles.

These earlier studies revealed that the host range of the several viruses showed considerable variation. Pea mosaic virus 2 infected *Phaseolus angularis* Wight, *Cicer arietinum* L., *Lathyrus odoratus*, *Lens esculenta* Moench, *Melilotus alba* Desr., *Trifolium repens*, and

Vicia faba. The white clover mosaic virus was infectious to all of these hosts except *P. angularis*, and in addition it infected *P. lunatus* L., *P. mungo* L., *Medicago sativa* L., *Trifolium pratense*, and *Vicia americana* Muhl. The white sweetclover virus was infectious to all of the hosts susceptible to the white clover virus and, in addition, to *Cajanus indicus* Spreng.; but it was not infectious to *P. lunatus* and *Trifolium repens*.

The virus of red clover mosaic was infectious to all the hosts that were susceptible to the white clover and white sweetclover viruses, with the exception of *Phaseolus lunatus*, *P. mungo*, *Cajanus indicus*, *Medicago sativa*, and *Melilotus alba*.

From the results just mentioned it is apparent that the various viruses tested have different host ranges. In general, the reaction of a given host to a number of viruses was the same as that expressed by symptomatology, but different hosts showed a variety of symptoms.

The writers (*14, 15*) also demonstrated that several of the legume viruses are infectious to bean, producing symptoms which make it possible to differentiate them from one another as well as from the common bean mosaic. Furthermore, differences in susceptibility and resistance among certain bean varieties were shown to be helpful in distinguishing the several viruses. In addition to studies on varietal resistance and susceptibility in the pea, preliminary separational reactions of certain bean varieties (table 2) and *Vicia faba* served to establish specific differences in the six viruses reported in this paper. Property studies of certain of the viruses discussed in this paper have been previously reported (*15*).

REACTION OF BEANS TO THE DIFFERENT VIRUSES

The mosaic viruses of pea and red clover can be distinguished from those of white clover and white sweetclover and from alsike clover mosaic virus 1 on the basis of their ability to infect bean (*Phaseolus vulgaris*). The viruses of pea and red clover are not infectious to bean, while the others produce definite symptoms on the Stringless Green Refugee variety.

The white clover mosaic virus is the only one that produces local lesions on bean (fig. 2, *H*) and thus it can be separated from the viruses of white sweetclover and alsike clover mosaic 1 (*15*). Furthermore, it produces systemic symptoms that differ from those produced by the other viruses. The symptoms on bean are very mild and are characterized by blotches of light green or yellow on the trifoliate leaves (fig. 2, *G*). Infected leaves may be slightly ruffled. However, there is no retardation in the growth of the leaf, and it is not malformed as it is when infected with the other viruses (fig. 2, *G*). Infected plants are never stunted.

The white sweetclover mosaic virus is readily distinguished from the others by the very marked symptoms that it produces on beans. These are first manifested by a drooping of the leaf and leaflets at the pulvini (fig. 3, *H*), followed by numerous small yellow halolike chlorotic spots (fig. 3, *I*). These spots enlarge and coalesce, producing a decidedly chlorotic condition of the leaf. The young trifoliate leaves are smaller than normal leaves and are decidedly rugose; they droop and curve inward, the tip in some cases almost touching the lower surface of the leaf (fig. 3, *H*). Infected plants are frequently stunted, and in severe cases they may die.

Alsike clover virus 1 produces only systemic lesions on beans, and thus it can be distinguished from the white clover mosaic, which it resembles. Furthermore, the systemic symptoms are more severe than those produced by the white clover virus and much less pronounced than those produced by the white sweetclover virus. They are first manifested by small light-green to yellow chlorotic spots, which enlarge and which may produce considerable chlorosis, especially on the older leaves. On the younger trifoliate leaves these spots frequently show normal green centers resembling a symptom of ring spot. Often light-green areas are found adjacent to the veins and veinlets with dark-green areas between, but occasionally the dark-green tissue may persist along the veins (fig. 4, *L*). Frequently large blotches of light green to yellow appear over the entire leaflet, resembling the symptoms produced by the white clover mosaic virus. Later, however, they are more marked and severe than those produced by the white clover virus and are easily differentiated. Infected leaves are somewhat smaller than normal and are slightly malformed, quite chlorotic, and somewhat rugose. Infected plants are only slightly stunted, the leaves and leaflets do not droop at the pulvini, nor do the young trifoliate leaves curve inward as they do in plants infected with the white sweetclover virus. From the last-named characteristic and from lack of severity of the symptoms, the alsike clover virus 1 can be identified as different from the white sweetclover virus. The fact that it does not produce local lesions and that it does cause characteristic light-green to yellow spots and severer and more pronounced symptoms serves as a means of differentiating it from the white clover virus.

In addition to the Stringless Green Refugee variety, three varieties, the Corbett Refugee, Robust, and Great Northern Idaho No. 1, which are resistant to the common bean mosaic virus, were used as a means of identifying the several viruses on the basis of varietal susceptibility and resistance. The results of these inoculations are shown in table 2.

Table 2 shows that the viruses of the common pea mosaic and the red clover mosaic are not infectious to bean. As mentioned earlier these two viruses can be separated from the others by this characteristic. The white clover mosaic virus, in addition to being the only one that produces local lesions on beans, can be separated from the other viruses by the fact that it is infectious to Stringless Green Refugee, Corbett Refugee, and Robust varieties, but not to Great Northern Idaho No. 1. The difference noted between alsike clover virus 1 and the white sweetclover virus is that the former is not as infectious as the latter, nor does it produce, except on the Stringless Green Refugee, as severe symptoms as does the white sweetclover virus.

TABLE 2.—*Resistance and susceptibility of 4 bean varieties to the mosaic viruses of several different legumes*

Variety	Reaction to indicated virus									
	Common pea mosaic		Red clover mosaic		White clover mosaic		White sweet-clover mosaic		Alsike clover mosaic	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
Stringless Green Refugee	12	0	10	0	12	11b	10	10a	16	9a, 3b, 4c
Corbett Refugee	12	0	11	0	15	2b, 7c	10	4a, 4b,	10	5b, 3c
Robust	10	0	10	0	10	3c	10	5b, 3c	18	2c
Great Northern Idaho No. 1	10	0	12	0	10	0	10	5b	24	1c

[1] *a* represents severe symptoms; *b*, moderate symptoms; and *c*, mild symptoms.

REACTIONS OF VICIA FABA TO THE DIFFERENT VIRUSES

Vicia faba is susceptible to all of the viruses discussed here. Although the symptoms vary less than they do on beans, a few of the viruses can be differentiated by differences in symptoms and also by severity of infection.

Of all the viruses those of the common pea mosaic and the red clover mosaic produce the severest symptoms on *Vicia faba*, and they cannot be differentiated from each other. The first symptom is a slight chlorosis of the affected plant. Islands of green tissue next appear adjacent to the veins and veinlets and frequently between them (fig. 1, *J*). Infected leaves may show considerable blistering and puckering in these regions and are often decidedly malformed. They are usually smaller than normal, and their edges are wavy and curl upward, especially toward the top of the plant. Severely infected plants are usually stunted and decidedly chlorotic.

The viruses of the mosaics of white clover, white sweetclover, and alsike clover 1 produce symptoms similar to one another when inoculated to *Vicia faba*. Infected plants are only mildly chlorotic and the leaves are slightly distorted or malformed. Islands of green tissue are found adjacent to the veins, but they are not so large, numerous, or extensive as they are on plants infected with the viruses of pea and red clover mosaic. Infected plants are only slightly stunted.

TRANSMISSION

SEED

Whether the virus of pea mosaic is transmitted through seed is still a moot question. Dickson (*2*) reported considerable seed transmission in certain varieties grown in the greenhouse. Out of a total of 130 seeds of 6 varieties, 55 produced diseased plants. Doolittle and Jones (*3*) reported no seed transmission in over 2,081 plants, which they grew from seed collected from mosaic-infected pea plants. Merkel (*5*) reported 0.8 percent of seed transmission. He did not state the number of seeds tested. Van der Meulen (*6*) was unable to show that the virus was carried through the seed. Böning (*1*) obtained only 0.2 percent of mosaic in a total of 9,180 plants of *Vicia*

faba grown from seeds from mosaic-infected plants.   Although it is assumed that the virus he worked with is identical with the mosaic reported by Doolittle and Jones (*3*), it cannot be said that seed transmission through *V. faba* is similar to that through the pea.   It appears that Dickson (*2*) may have been dealing with a different virus from those of other workers, because of the large amount of seed transmission he reports.

In order to secure additional evidence, seed from mosaic-infected pea plants, obtained from Colorado, Wyoming, Idaho, Montana, and Washington, was tested.   Seven varieties were represented.   Most of the seed was planted in the greenhouse, which was fumigated at regular intervals to prevent any secondary spread by insects.   The rest of the seed was planted in the field.   The infected seed collected in Montana was divided into two lots, (1) seed produced below the point of visible symptoms and (2) seed produced above this point.

Since it is possible to confuse primarily infected plants with those that may have become infected secondarily in the field, the notes were taken as soon as the symptoms appeared.   Primarily infected seedlings manifest the symptoms early by a mottling and a decided stunting.   When the first data were taken no mosaic had appeared in any of the lots except those that originated from infected plants. On the basis of this evidence it seems that the infected plants observed were produced from infected seed and were not infected from a secondary source.   Through cross-inoculation studies it was determined that the virus in question was that of the common pea mosaic. Table 3 shows the results of this study.

TABLE 3.—*Results from test on seed transmission of pea mosaic*

Variety	Seed from mosaic-infected plants grown in—	Where seed was planted	Plants produced	Plants infected with mosaic
			Number	*Number*
Alderman	Washington	Greenhouse	100	0
Green Giant	Montana	do	[1] 750	2
Do	do	Field	[1] 814	6
Do	do	Greenhouse	[2] 200	0
Do	do	Field	[2] 339	0
Dwarf Telephone	Colorado	do	118	3
Do	do	do	67	0
Potlatch (Improved Stratagem)	Idaho	Greenhouse	314	0
Rogers D	do	do	120	0
Dwarf Alderman	do	do	22	0
Prince of Wales	do	do	213	0
Total			3,057	11

[1] From seeds collected above point of noticeable infection.
[2] From seeds collected below point of noticeable infection.

In a total of 3,057 seedlings only 11 showed mosaic infection.   Out of a total of 1,564 plants from seeds produced above the point of noticeable infection, 8 plants showed the typical mosaic symptoms. Of 539 plants grown from seeds produced below the point of visible infection, no mosaic was observed.   From this limited evidence it appears that only seeds produced above the infection point may carry the virus.   Since the virus was transmitted through the seed in only a comparatively small percentage of cases, these results are not conclusive, but the evidence indicates that there is at least a

great reduction in primarily infected seeds if the seed is collected below the point of infection.

No data are available on seed transmission through peas of the other legume mosaic viruses to which peas are susceptible. In one trial of 140 plants from seeds collected from mosaic-infected plants in Maryland in 1932 and grown in the greenhouse, 25 plants showed mosaic symptoms. Inoculation to bean indicated that this virus was similar to pea mosaic virus 2, described in an earlier paper (*15*). Although this test is not conclusive, because of the small number of seeds tested, it does appear that some of these viruses may be carried through pea seed, possibly in higher percentages than the virus of the common pea mosaic. Because of the similarity of symptoms of these various viruses on pea, it would be difficult to determine a particular virus except through cross-inoculation.

INSECTS

The transmission of pea mosaic by the pea aphid (*Illinoia pisi*) was reported by Dickson (*2*), Doolittle and Jones (*3*), and Böning (*1*). Osborn (*7, 8*) transmitted a pea mosaic virus by means of the pea aphid (*Macrosiphum [Illinoia] pisi*) and the potato aphid (*M. gei*). He was not able to prove the bean aphid (*Aphis rumicis* L.) to be a vector. The writers were likewise able to transmit the pea and red clover mosaic viruses with the pea aphid, thus confirming the results of the previous workers. Transmission of the virus of white sweet-clover was secured with the pea and bean aphids, while tests with the green peach aphid (*Myzus periscae* Sulz.) gave negative results. In a few tests the pea aphid did not transmit the white clover mosaic virus to peas.

DISCUSSION

With the discovery of the transmissibility of various legume viruses to pea, the pea mosaic problem naturally becomes more complex. Although it had been shown prior to these investigations that a virus from red clover mosaic was transmissible to pea (*3*) and to several other legumes (*1, 2, 4*), and that a virus of pea mosaic is infectious to red clover (*3*), it had not been shown that several other legume viruses when inoculated to pea would produce infection. In fact, earlier tests had shown that a virus from *Melilotus alba* was not infectious to pea (*3*).

It is believed that the investigations presented here are significant insofar as they show that the pea can be infected with several distinct viruses producing symptoms which may be difficult to differentiate under certain environmental conditions. The results further show that the pea virus complex, unlike that of many other crops, may consist of distinct viruses of related hosts and not necessarily strains of the pea mosaic virus. If the several viruses described in this paper had been isolated from peas, one would have been led to believe that several strains or types of pea mosaic exist. It is not unlikely that later studies may show this to be the case. Doolittle and Jones (*3*) believe that aphids migrating from red clover to peas act as carriers of the disease, thus assuming that the red clover mosaic virus they worked with may have been identical with the common pea mosaic virus. In the inoculation studies of these investigations the red clover virus collected from numerous sources gave reactions identical

with the common pea mosaic virus except in the case of one collection, which will be discussed later. The symptoms produced by the other virus collections when inoculated to pea were identical with the symptoms of the common pea mosaic. Furthermore, both viruses produced identical symptoms on *Vicia faba* and neither was infectious to bean. More extended studies on host relationship and virus properties are necessary before it can be definitely said that these two viruses are identical. From preliminary evidence, however, it may be assumed that they are at least very closely related.

The question arises as to whether the virus from red clover here reported is a true mosaic virus of pea or of red clover. Since legume mosaics undoubtedly originated a long time ago, such a question is not susceptible to direct proof. However, since red clover is a winter-hardy perennial and the pea a semihardy annual, it would seem that a virus originating in red clover would have a much better opportunity of surviving than one occurring in peas. Moreover, red clover plants are only slightly injured by the virus while peas may be seriously injured, indicating perhaps that evolutionary tolerance has been developing for a longer time in the red clover than in the pea.

The other viruses considered in this paper have not been found as commonly on peas under natural conditions, indicating that peas are probably not a natural or original host. On the other hand, most of the numerous collections of viruses made from a given clover host were found to be a common virus of that host, showing a tendency for a virus to be fairly specific to the host in question.

It should not be understood that all the viruses collected from red clover are identical, even though the symptoms may indicate this. In one case it was found that a virus from this host produced symptoms on beans different from any reported here. Since other mosaic viruses such as the white clover, white sweetclover, alsike clover, and alfalfa are transmissible to *Trifolium pratense* (*15*), it is possible that certain of these viruses may be present in this host and that cross-inoculation studies with such material would reveal results different from those expected. However, from numerous tests made with material from a number of different sources it is believed that the typical red clover mosaic virus is not infectious to bean.

Even though white sweetclover is susceptible to the viruses of white clover, alsike clover, and alfalfa, only one type of symptoms has been produced on peas by the white sweetclover virus from many collections. On certain varieties of peas the symptoms manifest themselves very mildly and resemble those produced by the white clover mosaic virus. In the course of these experiments material from numerous sources was used. The mosaic virus of yellow sweetclover (*Melilotus officinalis* (L.) Lam.) was not used in these studies, but since its reaction on bean as reported earlier (*15*) was identical with that of white sweetclover, it is believed that its reaction on peas would also be similar.

Since the several viruses are intertransmissible between the various legumes, it is difficult to classify them according to the host from which they are collected. Likewise it is not always possible to separate all of them by means of the symptoms they produce on peas. Since these viruses when inoculated to certain varieties of beans and *Vicia faba* show differences, both as to symptoms and as to susceptibility and resistance, an additional means is available for differentiat-

ing them. Further studies on host range and virus properties, now in progress, may show still other distinguishing features.

As mentioned earlier, Böning's (*1*) virus disease of *Vicia faba,* which he transmitted to red clover, crimson clover, and pea, was believed by him to be probably identical with the red clover and pea mosaic reported by Doolittle and Jones *(3)*. Limited observations by the writers with a virus isolated from mosaic-infected *V. faba* seedlings grown from infected seed confirmed these results. It is also believed that these two viruses are probably identical with the mosaic viruses of pea and red clover reported here.

The streak disease of peas reported by Linford,[6] characterized by streaked and spotted brown necrosis of pods, stems, and leaves, is unlike any of the viruses discussed in this paper. Pierce's (*9*) bean virus 2 is very likely identical with the white sweetclover virus referred to here. Pierce's (*9*) alfalfa virus 2, which was reported as being infectious to pea and *Vicia faba* and the alfalfa mosaic described by Weimer (*12*) are dissimilar to the viruses dealt with here.

The pea mosaic which Osborn (*7*) described as producing leaf enations on both peas and crimson clover is different from any of the viruses here described. Osborn's (*8*) pea mosaic 2 appears to be similar to the viruses of the common pea mosaic and red clover mosaic discussed in this paper.

SUMMARY

Field surveys showed that pea mosaic is usually more severe along borders of fields than elsewhere, suggesting the possibility of the transmission of certain legume viruses to pea.

Cross-inoculation studies showed that the mosaic viruses of red clover, white clover, white sweetclover, and alsike clover are infectious to pea.

It is probable that other mosaic viruses may be found in the several legumes that may react differently from those described here, notwithstanding the similarity of symptoms.

Pea mosaic has been found wherever peas are grown, but the disease seems to be more serious in sections where the market-garden varieties are grown either for shipping as green peas or for seed.

Under certain environmental conditions and on certain varieties, the viruses of the several legumes may produce symptoms similar to those of the common pea mosaic. By the use of Dwarf Telephone, Tall Telephone, and Green Giant pea varieties, the mosaic viruses of white clover, white sweetclover, and alsike clover can be differentiated from the viruses of pea and red clover mosaics. The symptoms produced by the mosaic viruses of pea and red clover are similar, and it is believed that the two viruses are identical. In addition to the mottled symptoms produced by all of the legume viruses when inoculated to pea, the mosaic viruses of white clover and white sweetclover produced necrotic lesions on the inoculated leaves and stems.

Susceptibility and resistance to the several viruses were studied with 42 varieties of peas. Ten varieties were not infected by the pea mosaic virus and 8 were not infected by the virus of red clover mosaic. Three varieties out of 40 did not exhibit the mottled symptoms of the white clover virus, but all were susceptible to the necrotic lesions, only

1 variety showing a high degree of tolerance. The virus of white sweetclover mosaic produced the mottled symptoms on 38 varieties and necrotic lesions on 39. Seven varieties were not infected by alsike clover virus 1. The highest percentage of varieties not infected by the various viruses was found in the canning group.

In addition to the production of symptomatological differences, the several viruses can be separated on the basis of the reaction of Stringless Green Refugee, Corbett Refugee, Robust, and Resistant Great Northern Idaho No. 1 bean, and of *Vicia faba*.

Out of a total of 3,057 seeds collected from mosaic-infected pea plants, 11 produced diseased seedlings. In 1,564 plants grown from seeds collected above the point of noticeable infection, 8 were diseased, while in 539 seedlings grown from seeds collected below the point of noticeable infection no mosaic was observed.

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PARIS GREEN AND ITS HOMOLOGUES AS INSECTICIDES AGAINST THE JAPANESE BEETLE [1]

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INTRODUCTION

During the summer of 1934 an investigation was made to determine the value of paris green and certain of its homologues as stomach-poison insecticides against the adult Japanese beetle (*Popillia japonica* Newman).

The materials investigated are listed in table 1.[2] With the exception of paris green and copper crotonoarsenite, the materials were ground in a paint mill with bone glue, ethylene glycol, and ethyl alcohol to form a paste that would disperse readily in water. The paris green was passed five times through a colloid mill to obtain a fine dispersion in water. Copper crotonoarsenite did not require a wetting agent and, because of the small quantity available for experimentation, was ground with water in a mortar as required. The arsenious oxide and cupric oxide in these materials were determined by the regular procedure [3] and are given in table 1.

TABLE 1.—*Materials investigated and their arsenious oxide and cupric oxide content*

Material	Total As$_2$O$_3$ in dry material	CuO in dry material	Water-soluble As$_2$O$_3$ (dry basis)
	Percent	*Percent*	*Percent*
Fish-oil green	23.30	17.34	11.48
Soybean-oil green	23.68	19.02	1.15
Tung-oil green	29.84	19.89	.12
Rapeseed-oil green	30.28	19.76	7.53
Cottonseed-oil green	30.52	20.51	4.93
Copper stearoarsenite	31.38	20.72	4.29
Copper oleoarsenite	35.45	19.89	2.61
Paris green	35.52	29.90	1.94
Copper palmitoarsenite	38.59	22.10	4.88
Copper lauroarsenite	43.65	24.23	1.94
Copper crotonoarsenite	52.55	31.96	5.33

EXPERIMENTAL PROCEDURE

One liter of each spray was prepared to contain a definite quantity of the dry weight of paris green or one of its homologues, and placed in a glass tank of 1.5 liters capacity, which was equipped with a small mechanical agitator to keep the solids in suspension. The sprays contained 1.25, 2.5, 5, 10, 20, and 40 grams of dry material per liter, which were approximately equivalent to 1, 2, 4, 8, 16, and 32 pounds

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to 100 gallons. The spray flowed by gravity to a commercial spray gun and was applied to foliage under a 20-pound pressure. Smart-weed plants (*Polygonum pennsylvanicum*) in pots were placed on revolving disks, mounted on a revolving table, and passed slowly through a fan-shaped spray, so that each material was applied uniformly to the foliage.

After being sprayed the plants were allowed to dry and were then placed in special glass cages under controlled temperature, relative humidity, and light. The details of the environmental conditions and the procedure of testing stomach-poison insecticides on the Japanese beetle have been described by the writers.[4] In testing the insecticidal value of a definite concentration of a material, 1,000 freshly collected beetles that had been starved for 6 hours were distributed in five cages containing treated plants, 200 beetles in each cage; at the same time an equal number were confined with
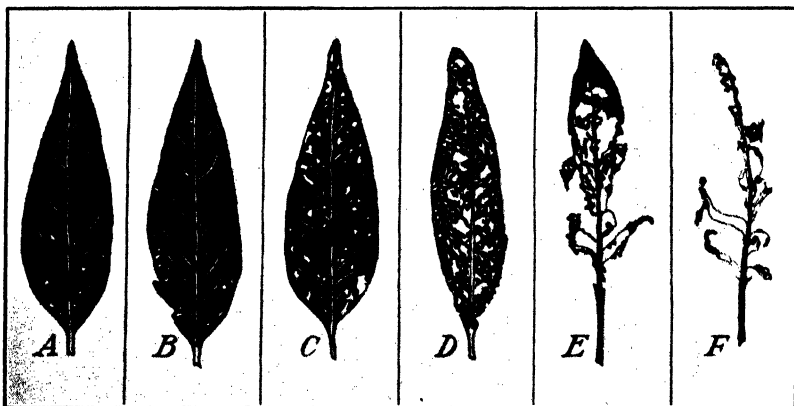

FIGURE 1.—The different degrees of feeding on foliage of smartweed by the Japanese beetle: *A*, Very slight; *B*, slight; *C*, moderate; *D*, extensive; *E*, very extensive; *F*, complete defoliation.

foliage sprayed with 8 pounds of acid lead arsenate and 2 pounds of light-pressed fish oil to 100 gallons of water to serve as a standard of insecticidal action, and an equal number were confined without food to determine the mortality caused by starvation. After 48 hours a record was made of the injury to the foliage caused by the spray material, the extent of feeding, and the number of beetles dead and alive in each cage.

The injury caused by a spray was considered to be "slight" when a few discolored spots appeared, "moderate" when these spots were small but generally distributed, "severe" when the surface of a large proportion of the leaves was injured, and "very severe" when all the foliage was damaged by the spray.

The extent of feeding by the beetles on smartweed foliage is indicated graphically in figure 1. The estimates on feeding are the averages obtained after a careful examination of all the foliage on the plants in the treatment.

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INSECTICIDAL VALUE

DETERMINATION OF COEFFICIENTS OF EFFECTIVENESS

The average mortality in the cages with the standard insecticide (acid lead arsenate), with each concentration of the different materials under test, and in the starvation cages was determined.   The average mortality attributed to poisoning by each concentration of a material was obtained by subtracting the average natural mortality in the starvation cages from the average mortality occurring in the treated cages. The effectiveness of each concentration of a material was expressed as a coefficient of the insecticidal action of the standard insecticide.[5] The coefficients of effectiveness of different concentrations of paris green and its homologues are given in table 2.

TABLE 2.—*Effectiveness of paris green and its homologues as stomach-poison insecticides and as repellents for the Japanese beetle, and their effect on foliage*

Material	Concentration	Coefficient of effectiveness [1]	Extent of injury by spray to foliage	Extent of feeding by beetles on sprayed foliage
	Pounds per 100 gallons			
Copper crotonoarsenite [2]	2	0.175±0.113	None	Extensive.
	4	.505± .138	Slight	Moderate.
	8	.807± .151	Moderate	Slight.
	16	.918± .090	Severe	Do.
Copper lauroarsenite [2][3]	2	.283± .092	Slight	Moderate.
	4	.302± .096	Moderate	Do.
	8	.387± .064	Severe	Slight.
Copper oleoarsenite	2	0	None	Defoliation.
	4	0	Very slight	Extensive.
	8	.156± .161	Slight	Moderate.
	16	.439± .179	Moderate	Slight.
	32	.622± .206	Very severe	Very slight.
Copper palmitoarsenite	1	0	None	Defoliation.
	2	0	do	Do.
	4	.458± .101	Slight	Moderate.
	8	.590± .108	do	Slight.
	16	.939± .231	Moderate	Do.
	32	1.097± .243	Very severe	Very slight.
Copper stearoarsenite	1	0	None	Defoliation.
	2	0	do	Do.
	4	0	do	Moderate.
	8	.108± .107	Very slight	Do.
	16	.378± .172	Slight	Slight.
	32	.609± .163	Moderate	Do.
Cottonseed-oil green	2	.427± .031	None	Moderate.
	4	.393± .114	Slight	Do.
	8	.473± .090	Moderate	Do.
	16	.625± .096	do	Slight.
Fish-oil green [3][4]	32	.833± .136	Severe	Very slight.
	4	.680± .065	Slight	Moderate.
	8	.546± .147	Moderate	Do.
Paris green	1	0	None	Defoliation.
	2	.311± .061	do	Moderate.
	4	.679± .065	Slight	Do.
	8	.698± .078	do	Slight.
	16	.753± .107	do	Do.
Paris green and flour in ratio 3 : 2	1	.076± .033	None	Extensive.
	2	.298± .050	do	Moderate.
	4	.359± .062	Very slight	Do.
	8	.633± .067	Moderate	Slight.

See footnotes at end of table.

[1] The standard error of each average and each difference was determined, and the error of the coefficient of effectiveness calculated according to the equation

$$\text{Error of quotient} = \pm \frac{P}{S}\sqrt{\frac{p^2}{P^2} + \frac{s^2}{S^2}}$$

where P is the number of beetles poisoned by the material, S is the number poisoned by the standard insecticide, and p and s are the respective standard errors.

TABLE 2.—*Effectiveness of paris green and its homologues as stomach-poison insecticides and as repellents for the Japanese beetle, and their effect on foliage*—Contd.

Material	Concentration	Coefficient of effectiveness	Extent of injury by spray to foliage	Extent of feeding by beetles on sprayed foliage
	Pounds per 100 gallons			
	1	0	None	Extensive.
	2	0	do	Moderate.
Paris green and summer oil (summer oil 1 pint to 100 gallons).	4	.291± .061	Very slight	Do.
	8	.417± .070	Slight	Slight.
	16	.842± .074	Moderate	Very slight.
	32	1.039± .083	do	Do.
	2	0	None	Defoliation.
Rapeseed-oil green [2]	4	0	do	Do.
	8	.167± .077	Moderate	Moderate.
	16	.094± .072	Severe	Slight.
	4	.191± .040	None	Moderate.
Soybean-oil green	8	.232± .099	Slight	Do.
	16	.420± .101	Moderate	Slight.
	32	.526± .126	Severe	Very slight.
	2	.136± .075	None	Extensive.
	4	.339± .116	Slight	Moderate.
Tung-oil green	8	.562± .133	do	Do.
	16	.830± .089	Moderate	Slight.
	32	.913± .167	Severe	Very slight.

[1] As compared with acid lead arsenate=1.000±0.060.
[2] The injury was so severe at the highest concentration recorded that further tests were not made.
[3] The homologues of paris green, particularly copper laurcarsenite and fish-oil green, when suspended in water had some coarse particles which clogged the fine nozzle of the spray gun.
[4] It was not possible to determine the values of the coefficients of fish-oil green accurately because of the variability of the data.

RELATION BETWEEN THE COEFFICIENT OF EFFECTIVENESS AND THE CONCENTRATION

When the coefficients of effectiveness of the various materials were plotted against the concentrations, a logarithmic curve was obtained. To determine accurately the position of this line, the constants of the equation of the line were determined mathematically by the method of least squares.[6] It was found that the values obtained from the equation $Y'=a+b \log X$, where Y' is the estimated coefficient of effectiveness, $\log X$ is the logarithm of the concentration, and a and b are constants, came the closest to the determined values of the coefficients. It should be noted that this equation will not give the effectiveness at zero concentration, which, of course, is zero, but there appears to be a close agreement between the estimated and the determined values when the materials were used at the rate of 8 pounds to 100 gallons, at which concentration the effectiveness of the materials was compared. The index of correlation, the index of determination, and the standard error of estimate were then calculated.[6] These values are given in table 3.

There is a high correlation between the coefficient of effectiveness and the concentration of all materials, except in the case of rapeseed-oil green. The index of determination shows what proportion of the variance in the dependent variable can be explained by the concomitant variation in the independent variable. It was found that, with the exception noted, more than 75 percent of the variance in the coefficients of effectiveness could be accounted for by the change in the concentration. Since this leaves only a small percentage of the variance to be accounted for by all other factors, it appears that the concentration was the most important factor associated with the coefficient of effectiveness of these materials.

[6] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. chs. 7–8. New York and London. 1930.

TABLE 3.—*Statistical measures showing the association between the coefficients of effectiveness of paris green and its homologues and their concentration in the spray*

Material	Index of correlation	Index of determination	Standard error of estimate [1]
		Percent	
Tung-oil green	0.9876	0.9754	0.0501
Copper palmitoarsenite	.9751	.9508	.1020
Copper crotonoarsenite	.9696	.9402	.0816
Paris green and summer oil	.9656	.9324	.1121
Paris green and flour	.9628	.9270	.0619
Soybean-oil green	.9625	.9263	.0429
Copper oleoarsenite	.9455	.8939	.0903
Paris green	.8964	.8036	.1437
Cottonseed-oil green	.8789	.7724	.0865
Copper lauroarsenite	.8737	.7633	.0269
Copper stearoarsenite	.8706	.7579	.1255
Rapeseed-oil green	.5174	.2677	.0693

[1] In terms of the coefficient of effectiveness.

The standard error of estimate may be used as a measure of the consistency of the insecticidal action of a material. Copper lauro-arsenite, soybean-oil green, and tung-oil green were the least variable and copper stearoarsenite was the most variable of the homologues of paris green, but the last material was less variable than paris green. When flour or summer oil was mixed with paris green, the variation in the insecticidal results with this material was reduced.

Theoretically, when the linear correlation is high, the averages of several observations for each concentration of a material should lie along the regression line, and these averages should coincide with the estimated values. An examination of the different regression lines showed that the experimentally determined values were arranged about the lines but did not always coincide. It is to be expected in insecticidal tests, even under controlled conditions, that there will be some fluctuation in the determined values. To establish these points more definitely it would be necessary to make many more tests than was possible during the limited period in the summer when the adult beetle is available for experimentation. It is believed that under the circumstances the values for a given concentration obtained from the regression line probably afford a better basis of comparison than the determined values. It was decided, therefore, to use the estimated values of the coefficients of effectiveness at a concentration of 8 pounds to 100 gallons for determining the relative effectiveness of paris green and its various homologues.

As these estimated coefficients of effectiveness are subject to variation, as are other statistical constants, the standard errors of the coefficients were determined by the procedure outlined by Ezekiel.[7] The coefficients were than arranged in order of increasing difference from the coefficient of acid lead arsenate, and the significance of the difference was determined. These data are given in table 4.

[7] EZEKIEL, M. See p. 253 of reference cited in footnote 6.

TABLE 4.—*Comparison of the coefficients of effectiveness of lead arsenate and paris green and its homologues*

Material	Coefficient of effectiveness [1]	Comparison of the coefficients of effectiveness of material indicated with coefficients of less effective materials shown in first column			
		Acid lead arsenate		Copper crotonoarsenite	
		Difference	Difference÷error	Difference	Difference÷error
Acid lead arsenate	1.000±0.060				
Copper crotonoarsenite	.728± .045	0.272±0.075	3.63		
Paris green	.677± .079	.323± .099	3.26	0.051±0.091	0.56
Copper palmitoarsenite	.635± .043	.365± .074	4.93	.093± .062	1.50
Paris green and flour	.601± .052	.399± .079	5.05	.127± .069	1.84
Tung-oil green	.556± .023	.444± .064	6.94	.172± .051	3.37
Cottonseed-oil green	.550+ .039	.450± .072	6.25	.178± .060	2.97
Paris green and summer oil	.544± .048	.456± .077	5.92	.184± .066	2.79
Copper lauroarsenite	.376± .024	.624± .065	9.60	.352± .051	6.90
Soybean-oil green	.283± .023	.717± .064	11.20	.445± .051	8.73
Copper stearoarsenite	.244± .053	.756± .080	9.45	.484± .070	6.91
Copper oleoarsenite	.243± .040	.757± .072	10.51	.485± .060	8.08
Rapeseed-oil green	.088± .038	.912± .071	12.85	.640± .059	10.85

Material	Comparison of the coefficients of effectiveness of material indicated with coefficients of less effective materials shown in first column					
	Tung-oil green		Copper lauroarsenite		Soybean-oil green	
	Difference	Difference÷error	Difference	Difference÷error	Difference	Difference÷error
Cottonseed-oil green	0.006±0.045	0.13				
Paris green and summer oil	.012± .053	.23				
Copper lauroarsenite	.180± .034	5.29				
Soybean-oil green	.273± .034	8.03	0.093±0.034	2.74		
Copper stearoarsenite	.312± .058	5.38	.132± .059	2.24	0.039±0.058	0.67
Copper oleoarsenite	.313± .046	6.80	.133± .047	2.83	.040± .046	.87
Rapeseed-oil green	.468± .044	10.64	.288± .045	6.40	.195± .044	4.43

[1] Determined for 8 pounds of material from the regression equation $Y''=a+b \log X$.

It is generally accepted that a value may be considered significant when it is at least two times its standard error. By this criterion of significance, acid lead arsenate was found to be significantly more effective as an insecticide than paris green or any of its homologues. Copper crotonoarsenite, which was about 73 percent as effective as the standard, was not significantly different from copper palmitoarsenite, paris green, and paris green and flour. It was not possible to demonstrate significant differences between tung-oil green, which was about 56 percent as effective as the standard, and cottonseed-oil green, or paris green with summer oil. Copper lauroarsenite, which was about 38 percent as effective as the standard, was significantly less effective than tung-oil green and significantly more effective than soybean-oil green, copper stearoarsenite, copper oleoarsenite, and rapeseed-oil green. Soybean-oil green was not significantly more effective than copper stearoarsenite and copper oleoarsenite. Rapeseed-oil green was significantly less effective than the other materials and was only about 9 percent as effective as acid lead aresenate.

The coefficient of regression measures the slope of the regression line; it shows, in the present case, the average change in the coefficient of effectiveness with each unit increase in the logarithm of the concentration. The variation likely to be present in the coefficient of regression of each material was determined by the procedure outlined by Ezekiel.[8] The coefficients of regression were arranged in order of decreasing magnitude and the significance of the differences between them was determined, as given in table 5.

TABLE 5.—*Significance of difference in change in effectiveness of paris green and its homologues with change in concentration of the spray as shown by their regression coefficients*

Material	Coefficient of regression	Difference between coefficient of regression of indicated material and those of less rapidly changing materials shown in the first column			
		Copper crotonoarsenite		Copper stearoarsenite	
		Difference	Difference ÷ error	Difference	Difference ÷ error
Copper crotonoarsenite	0. 8408±0. 1212				
Copper palmitoarsenite	. 8005± . 0810	0. 0403±0. 1458	0. 28		
Paris green and summer oil	. 7448± . 0890	. 0960± . 1504	. 64		
Tung-oil green	. 6793± . 0537	. 1615± . 1326	1. 22		
Paris green	. 6289± . 1509	. 2119± . 1935	1. 09		
Paris green and flour	. 5753± . 0924	. 2655± . 1524	1. 74		
Copper oleoarsenite	. 5591± . 0949	. 2817± . 1539	1. 83		
Copper stearoarsenite	. 4069± . 0997	. 4339± . 1569	2. 77		
Soybean-oil green	. 3963± . 0637	. 4445± . 1369	3. 25	0. 0106±0. 1183	0. 09
Cottonseed-oil green	. 3468± . 0908	. 4940± . 1514	3. 26	. 0601± . 1348	. 45
Copper lauroarsenite	. 1727± . 0633	. 6681± . 1367	4. 89	. 2342± . 1181	1. 98
Rapeseed-oil green	. 1492± . 1030	. 6916± . 1591	4. 35	. 2577± . 1433	1. 80

Copper crotonoarsenite, which increased in effectiveness at the average rate of about 84 percent of the logarithm of the concentration, did not differ significantly in its rate of change from copper palmitoarsenite, tung-oil green, copper oleoarsenite, or paris green, alone or mixed with flour or summer oil. Copper stearoarsenite changed in effectiveness at the rate of about 41 percent of the logarithm of the concentration and did not differ significantly from soybean-oil green, cottonseed-oil green, copper lauroarsenite, and rapeseed-oil green.

EFFECT OF QUANTITY OF RESIDUE ON FOLIAGE

The residues on the foliage were most conspicuous with those materials having a high coefficient of regression. Although no chemical analysis was made of the sprayed foliage to determine the effect of the change in concentration on the quantity of residue, it is apparent that the quantity deposited on the foliage must have increased most rapidly with materials having a high coefficient of regression.

EFFECT OF ARSENIOUS OXIDE CONTENT

It was believed that there might be some correlation between the effectiveness of paris green and its homologues as insecticides and the percentage of the total or water-soluble arsenious oxide in the compounds. As no correlation was found, it is apparent that factors other than the arsenious oxide content are more important in modifying the insecticidal action of these materials.

[8] EZEKIEL, M. See p. 252 of reference cited in footnote 6.

CONCLUSION AS TO INSECTICIDAL EFFECTIVENESS

It appears from these several considerations that copper crotono-arsenite, which was 73 percent as effective as acid lead arsenate when used at a concentration of 8 pounds to 100 gallons, and which increased in effectiveness at the rate of 84 percent of the logarithm of the concentration, was the most promising in this group of materials as an insecticide.

FEEDING BY BEETLES ON SPRAYED FOLIAGE

The Japanese beetle is an intermittent feeder, moving from one part of the foliage to another and making several small punctures, such as those shown in figure 1, *A* and *B*. When the foliage is palatable and several beetles are present, these punctures increase in number and size until all the tissue between the heavy veins of a leaf has been consumed. With a gregarious insect such as the Japanese beetle feeding in this manner, it is not possible to determine accurately the area of a leaf consumed by one beetle, but only to make a general estimate of the extent of feeding.

It was assumed that, for a material to be of value in protecting foliage from injury, the feeding should result in not more than a few small punctures at the end of the test period. This condition was obtained when copper crotonoarsenite, copper lauroarsenite, copper palmitoarsenite, or paris green, alone or in combination with flour or summer oil, was used at the rate of 8 pounds to 100 gallons, but the other arsenites had to be used at the rate of 16 pounds or more to give this degree of protection. It is apparent, however, that factors other than the toxicity of the material deter the beetles from feeding, for copper lauroarsenite, which is significantly less effective than copper crotonoarsenite as an insecticide, was equally as effective in making the foliage unpalatable.

INJURY BY THE MATERIALS TO FOLIAGE

It has been repeatedly observed that injury by spray materials to the foliage occurs more rapidly under conditions of high temperature and humidity, such as prevailed in these tests, than under other conditions. It might be concluded, therefore, that the conditions of these tests are the most exacting in determining the tolerance of foliage to the chemicals.

In each case the materials caused some injury to the foliage at a concentration lower than that which deterred the beetles from feeding. It is believed, therefore, that paris green and its homologues cannot be used in their present form to protect foliage from attack without the possibility of injuring the foliage.

SUMMARY

A study has been made of the relative effectiveness of paris green and its homologues as stomach-poison insecticides against the adult Japanese beetle (*Popillia japonica* Newman). The experiments were conducted under conditions in which the temperature, relative humidity, light, and food were controlled and beetles from the same source were used. The effectiveness of the materials as stomach

poisons was determined by comparison with acid lead arsenate applied to foliage at the rate of 8 pounds to 100 gallons of water and tested under the same conditions, and was expressed as the coefficient of the insecticidal action of the lead arsenate.

It was found that, except in the case of rapeseed-oil green, there was a high correlation between the coefficient of effectiveness of a material and its concentration. In general, 75 percent or more of the variance in the coefficients of effectiveness could be accounted for by the change in the concentration of the materials.

Acid lead arsenate was significantly more effective as an insecticide than paris green or its homologues. Copper crotonoarsenite, which was about 73 percent as effective as lead arsenate, was not significantly different in effectiveness from paris green or copper palmitoarsenite. It was not possible to demonstrate significant differences between the coefficients of effectiveness of tung-oil green and cottonseed-oil green. Copper lauroarsenite was about 38 percent as effective as the standard, and was significantly less effective than tung-oil green and significantly more effective than soybean-oil green, copper stearoarsenite, copper oleoarsenite, and rapeseed-oil green. Soybean-oil green was not significantly more effective than copper stearoarsenite and copper oleoarsenite. Rapeseed-oil green was significantly less effective than the other materials and was only about 9 percent as effective as acid lead arsenate.

Copper crotonoarsenite increased in effectiveness at the average rate of about 84 percent of the logarithm of the concentration and did not differ significantly in its rate of change from copper palmitoarsenite, tung-oil green, copper oleoarsenite, or paris green, alone or mixed with flour or summer oil. Copper stearoarsenite changed in effectiveness at the rate of about 41 percent of the logarithm of the concentration and did not differ significantly from soybean-oil green, cottonseed-oil green, copper lauroarsenite, and rapeseed-oil green.

There was no correlation between total or water-soluble arsenious oxide content and the effectiveness of the materials.

It was necessary to use copper crotonoarsenite, copper lauroarsenite, copper palmitoarsenite, and paris green, alone and in combination with flour or summer oil, at the rate of 8 pounds to 100 gallons to reduce the feeding by 200 beetles to only a few punctures on the foliage. The other materials had to be used at a concentration of 16 pounds or more to obtain this condition. As copper lauroarsenite was significantly less effective than copper crotonoarsenite as an insecticide but equally as effective in making the foliage unpalatable to the beetles, it is apparent that factors other than toxicity deter the beetles from feeding.

As some injury to foliage was caused by paris green and its homologues at lower concentrations than those that deterred the beetles from feeding, it is believed that these materials cannot be safely applied in their present form to control the beetle in the field.

Copper crotonoarsenite was the most promising of the materials studied as an insecticide for control of the Japanese beetle.

DERRIS AS A JAPANESE BEETLE REPELLENT AND INSECTICIDE [1]

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INTRODUCTION

It has been known for several years that the Japanese beetle (*Popillia japonica* Newm.) does not feed readily on foliage completely covered with lead arsenate, lime, and similar materials (*2, 3, 9, 10, 13, 16, 19*).[2] In 1932 and 1933 (*17*) it was found that spraying with suspensions of derris or rotenone at weekly intervals and after every rain protected foliage from severe injury by the beetle. The use of these sprays has the added advantage that the residue left on the plants is not conspicuous and is probably not harmful to man or animals.

It is difficult, however, to establish definitely the value of a material under field conditions, because of the many variable factors. Some orchards are heavily infested with beetles one season and lightly infested the next. Some orchards are heavily infested early in July and few beetles are present 2 or 3 weeks later; other orchards may not become heavily infested until late in July. The timeliness of the application of a spray is important. When spraying is delayed until beetles have become established on the plants, it is difficult to protect the plants from injury. Many materials that are effective against a light infestation have been found to be inadequate when beetles are very abundant. In view of these variable factors, it was decided to study derris and its components [3] under artificially controlled conditions of heavy infestation where the beetles were stimulated to feed.

DERRIS, ROTENONE, AND DEGUELIN AS CONTACT INSECTICIDES

It has been observed frequently in the field that the application of derris or rotenone sprays to a tree on which beetles are feeding causes large numbers to drop to the ground, and many of these paralyzed beetles subsequently die. A series of laboratory tests was conducted to determine the relative effectiveness of derris and two of its components, rotenone and deguelin, as contact insecticides, as compared with neutral potassium oleate, a material recognized as being effective as a contact insecticide against this insect.

The tests were conducted according to the procedure outlined by Fleming and Baker (*5*). The derris, rotenone, and deguelin were mixed with acetone at the rate of 4 g per 100 cc and added to water so that the concentration ranged from 0.5 to 20 g per liter, which was equivalent to 0.4 to 16 pounds per 100 gallons. These suspensions were kept homogeneous during the treatment by constant stirring.

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[1] Received for publication Apr. 9, 1936; issued September 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 206.
[3] The derris, containing 4 percent of rotenone, and the rotenone, 90 percent pure, were purchased on the open market; the deguelin, tephrosin, toxicarol, and derris resin, a material from which all crystalline substances had been removed, were prepared by E. P. Clark. of the Division of Insecticide Investigations.

Neutral potassium oleate soap, which was used as the standard insecticide, was prepared in concentrations ranging from 1.25 to 20 g of anhydrous soap per liter. Five groups, each of 100 beetles, were submerged for 120 seconds in each suspension at a temperature of 80° F. and then placed in cages held at 80° and a relative humidity of 96 to 100 percent for 24 hours to determine the effect of each treatment. Equal numbers of untreated beetles were placed in the cages to determine the natural mortality.

The average survival of 100 beetles treated with each concentration of the materials under test, and with the standard insecticide, and the average survival of the untreated beetles, with their respective standard errors, were determined. Since the mortality of the untreated beetles was assumed to be the natural mortality of the beetles used in the tests, one-half the survival among these beetles was selected as the median point where 50 percent were killed by the insecticidal treatment. The median effective concentration was then calculated according to the equation given by Fleming and Baker (7). The average survival of 100 beetles with each concentration of the materials and with their respective standards, the median effective concentrations, and the coefficients of effectiveness are given in table 1.

TABLE 1.—*Effectiveness of derris, rotenone, and deguelin as contact insecticides against the Japanese beetle as compared with neutral potassium oleate as the standard*

Material	Concentration	Average survival of 100 beetles		Median effective concentration		Coefficient of effectiveness
		Material	Standard	Material	Standard	
	Grams per liter	*Number*	*Number*	*Grams per liter*	*Grams per liter*	
Rotenone	0.00	97±0.83	97±0.83	0.717±0.060	1.651±0.060	2.302±0.210
	.50	72±4.97	--------			
	.75	45±4.30	--------			
	1.00	26±3.05	--------			
	1.25	26±5.68	70±2.99			
	2.50	1±.58	33±2.59			
	5.00	0	17±1.36			
	10.00	0	1±.77			
Deguelin	0.00	95±.55	95±.55	2.537±.137	2.537±.099	1.000±.067
	1.25	74±3.20	81±3.20			
	2.50	48±1.79	48±1.30			
	5.00	15±2.78	15±.97			
	10.00	0	2±.80			
	20.00	0	0			
Derris	0.00	95±.55	95±.55	6.560±2.364	2.537±.099	.387±.140
	1.25	83±2.07	81±3.20			
	2.50	70±3.88	48±1.30			
	5.00	50±3.42	15±.97			
	10.00	42±3.84	2±.80			
	20.00	8±2.12	0			
Derris with equal weight of castor oil.	0.00	97±.83	97±.83	2.968±.274	1.651±.060	.556±.055
	.31	84±1.22	--------			
	.62	66±2.48	--------			
	1.25	64±1.36	70±2.99			
	2.50	56±4.20	33±2.59			
	5.00	16±3.40	17±1.36			
	10.00	0	1±.77			
Derris with 0.125 percent potassium coconut-oil soap	0.00	89±1.22	89±1.22	1.239±.225	2.188±.135	1.766±.339
	1.00	50±4.84	73±3.44			
	2.00	27±5.31	50±3.58			
	3.00	21±2.17	21±2.42			
	4.00	11±2.76	--------			

In the tests in which derris and deguelin were used at the rate of 20 g per liter, half of the liquid was acetone. However, as the survival after treatment with acetone at this concentration was 85 ± 1.50,

it is apparent that the acetone did not appreciably influence the insecticidal results. Statistically there is no significant difference between the coefficients of derris alone and of derris mixed with castor oil, but when derris was added to a 0.125-percent solution of potassium coconut-oil soap, which alone did not kill beetles, the effectiveness was increased to 1.765 times the standard, which was not significantly different from that of rotenone.

These data suggest that if a small quantity of soap is added to the derris spray in the field the effectiveness of the material as a contact insecticide is increased.

DERRIS AND ITS COMPONENTS AS STOMACH-POISON INSECTICIDES AND REPELLENTS

A study was also made of the relative effectiveness of derris and its components as stomach poisons and repellents against the Japanese beetle. The derris was mixed with water at the rate of 250 g to 750 cc of water and passed five times through a colloid mill to obtain a finely divided suspension. The deguelin, rotenone, tephrosin, toxicarol, and derris resin were mixed with acetone at the rate of 2 g to 100 cc. The materials were added to water to obtain sprays containing 1.25, 2.5, 5, 10, 20, and 40 g per liter, which was approximately equivalent to 1, 2, 4, 8, 16, and 32 pounds to 100 gallons. A spray containing 10 g of acid lead arsenate and 2.5 g of light-pressed fish oil per liter was used as a standard insecticide.

The details of the environmental conditions and the procedure for testing stomach-poison insecticides on the Japanese beetle have been described by the authors (*4*, *6*). The method used in applying these materials is outlined in the paper on paris green and its homologues as insecticides against the Japanese beetle (*8*).

The average mortality obtained in cages with the standard insecticide (acid lead arsenate), with each concentration of the various materials under test, and in the starvation cages, together with their respective errors, was determined. The average mortality attributed to poisoning by each concentration of a material was obtained by subtracting the average natural mortality in the starvation cages from the average mortality due to the insecticidal treatments. For this difference the standard error was computed. The effectiveness of each concentration was expressed as a coefficient of the insecticidal action of the standard insecticide by dividing the average number of beetles poisoned by the average number poisoned in the standard.

The coefficients of effectiveness of derris and its components, and the extent of feeding by the beetles on the sprayed foliage, are given in table 2.

It was found that derris and its components, with the possible exception of rotenone, were of little value as stomach-poison insecticides. This confirms the results of previous investigations (*6*) in which it was shown that derris is only a weak stomach poison against the Japanese beetle. There was no injury to the foliage due to these sprays.

The beetles fed to only a limited extent on foliage sprayed with derris, even when the material was used at the rate of 2 pounds to 100 gallons. It is apparent that the repellent action of derris can be attributed to the rotenone and deguelin, as the toxicarol, tephrosin, and the derris resin had little deterrent effect on the insect.

TABLE 2.—*Effectiveness of derris and its components as stomach poisons and repellents against the Japanese beetle as compared with the standard, acid lead arsenate*

Material	Concentration	Coefficient of insecticidal effectiveness	Extent of feeding by the beetle on smartweed foliage.
	Pounds per 100 gallons		
Derris	1	0	Moderate.
	2	0	Light.
	4	0	Very light.
	8	0	Do.
	16	0	Do.
	32	0	Do.
Rotenone	1	0	Do.
	2	0.438±0.044	Do.
	4	.534± .038	Do.
Deguelin	1	0	Light.
	2	0	Do.
	4	0	Very light.
Toxicarol	1	0	Extensive.
	2	0	Do.
	4	0	Moderate.
	8	0	Do.
Tephrosin	1	0	Complete defoliation.
	2	0	Do.
	4	0	Extensive.
	8	0	Do.
Derris resin	1	0	Complete defoliation.
	2	0	Extensive.
	4	0	Moderate.
	8	0	Do.
Acid lead arsenate	8	1.000± .029	Light.

DERRIS AND OTHER MATERIALS AS REPELLENTS WHEN APPLIED TO HEAVILY INFESTED APPLES

Apples and peaches that ripen during the period when the beetles are numerous are not easily protected by the application of repellent sprays, because the attractiveness of the fruit overcomes to a large extent the effect of the spray. A study was made of the effectiveness, under controlled laboratory conditions, of various repellent sprays that had been found to be effective under favorable conditions in the field.

Twigs of apple of the variety Yellow Transparent were placed in 3-inch earthen pots of soil. The surface of the soil was covered with plaster of paris to hold the twigs firmly in position. Various sprays of lime, acid lead arsenate, rotenone, and derris, in most cases in combination with a sticker, were applied to the twigs under a pressure of 20 pounds per square inch. During this application apples that had been cut in half to make them more attractive to the beetles were fastened on wires and held in the different sprays until thoroughly coated. When the spray deposit was dry on the fruit and foliage, two half apples were suspended on each twig and placed in the special glass cages under controlled conditions. Three twigs were placed in each cage and five cages were used for each treatment. Then 200 freshly collected beetles, which had been starved for 6 hours, were liberated in each cage. After 48 hours a record was made of the extent of feeding by the beetles. A summary of these results is given in table 3.

TABLE 3.—*Effectiveness of derris, rotenone, hydrated lime, and acid lead arsenate as repellents to the Japanese beetle when applied to Yellow Transparent apples*

Material and sticker	Concentration		Extent of feeding by beetles—	
	Material	Sticker	On the fruit	On the foliage
	Pounds per 100 gallons	*Pounds per 100 gallons*		
No treatment....			Very extensive.	Very extensive.
Derris with light-pressed fish oil	3	0	None	None.
	3	1	do	Do.
	4	2	do	Do.
	8	2	do	Do.
Rotenone	4	0	do	Do.
	8	0	do	Do.
Hydrated lime	32	0	Extensive	Moderate.
Hydrated lime with light-pressed fish oil	20	1	do	Do.
Hydrated lime with flour	20	3	do	Do.
Hydrated lime with aluminum sulphate	20	3	do	Do.
Acid lead arsenate with light-pressed fish oil	6	1.5	Slight	None.
	8	2	do	Do.
Acid lead arsenate with flour	6	4	do	Do.

It was found under these artificial conditions of heavy infestation, where the beetles were stimulated to feed and had no choice between sprayed and unsprayed plants, that the application of lime at the rate of 32 pounds to 100 gallons, or mixed with fish oil, flour, or aluminum sulphate and used at the rate of 20 pounds to 100 gallons, did not protect the fruit from injury, although the feeding on the foliage was decreased. Acid lead arsenate, mixed with fish oil or with flour, protected the foliage but did not prevent the beetles from feeding to a slight extent on the fruit. Derris, alone or with fish oil, and rotenone afforded complete protection to the fruit and foliage.

The effect of lime and of acid lead arsenate under these artificial conditions was inferior to that usually obtained under conditions of moderate infestation in the field, which might be attributed to the fact that the beetles had no choice between sprayed and unsprayed plants. The results with derris and with rotenone were superior to those obtained in the field, probably because under the artificial conditions the materials were not subject to decomposition by sunlight or to mechanical loss by wind and rain.

EFFECT OF EXPOSURE TO ULTRAVIOLET LIGHT ON EFFECTIVENESS OF DERRIS AND ROTENONE

There are several statements in the literature that derris and rotenone are altered by exposure to light. Davidson and Jones (1), Jones and Haller (15), Gersdorff (11), and Tattersfield and Roach (18) have demonstrated that derris and rotenone in solution lose toxicity. Tests by Newcomer, Hough, and Lathrop against the codling moth and by Ginsburg against the silkworm [4] indicate that spray deposits lose much of their effectiveness after several days' exposure to sunlight. Jones, Gersdorff, Gooden, Campbell, and Sullivan (14) have shown that derris and rotenone are rapidly decomposed by direct sunlight, with a resulting decrease in toxicity to insects. It was observed during the seasons of 1932, 1933, and 1934 that deposits of derris and rotenone

[4] CAMPBELL, F. L. REVIEW OF INFORMATION ON THE INSECTICIDAL VALUE OF ROTENONE. U. S. Dept. Agr., Bur. Ent. E-298, 28 pp. 1932. [Mimeographed.]

on the foliage lost much of their effectiveness as repellents to the Japanese beetle 3 or 4 days after application, but it was not known whether this loss was due to weathering or to a chemical change in the material.

Suspensions of derris, rotenone, and deguelin were applied to the foliage of smartweed (*Polygonum pennsylvanicum*).   The sprayed plants were then set on a slowly rotating table and passed under the hood of a horizontal quartz mercury-vapor arc, placed 18 inches above the plants in such a manner that the light was directed on the foliage.   The radiation of this lamp extended from the 10,140 angstrom line in the infrared to the region of 1,850 angstrom in the ultraviolet.   Groups of plants were exposed for periods of 1, 12, and 24 hours and were then placed in cages with beetles.   Unfortunately, the exposure of the plants to the ultraviolet light caused a loss of turgidity and browning of the foliage; so it was impossible to determine definitely the reaction of the beetles.

In view of the adverse effect of ultraviolet light on the plants, it was decided to expose derris and rotenone to the light before applying it to the foliage.   Each material was made into a thin paste with acetone (20 g of material to 100 cc of acetone) and painted on glass plates, a margin being left around the edge to prevent loss in handling. The materials were then exposed for periods of 1 and 24 hours.   At the end of the exposure, the deposits were reground in a mortar and applied to smartweed foliage.   The reaction of the beetles to the plants sprayed with these materials is given in table 4.

TABLE 4.—*Effect of exposure to ultraviolet light on derris and rotenone as repellents and stomach poisons against the Japanese beetle*

Material	Concentration	Exposure to ultraviolet light	Coefficient of insecticidal effectiveness	Extent of feeding on smartweed foliage
	Pounds per 100 gallons	*Hours*		
	4	None	0	Very light.
		1	0	Moderate.
		24	0	Do.
	8	None	0	Very light.
Derris		1	0	Light.
		24	0	Do.
	16	None	0	Very light.
		1	0	Light.
		24	0	Do.
	4	None	0. 534±0. 038	Very light.
		1	0	Do.
		24	0	Light.
	8	None	. 477± . 040	Very light.
Rotenone		1	0	Do.
		24	0	Light.
	16	None	. 584± . 056	Very light.
		1	. 230± . 046	Do.
		24	0	Light.

It was not possible to detect the change in effectiveness of derris as a stomach poison after exposure to ultraviolet light, because of the low toxic value of the material.   Rotenone, on the other hand, was definitely decreased in toxicity.   When used at the rate of even 16 pounds to 100 gallons, it lost all its toxic value as a stomach poison after exposure to ultraviolet light for 24 hours.

The value of these materials as repellents was only slightly reduced by exposure to ultraviolet light for 24 hours, indicating that their repellent effect is not destroyed so rapidly as is their toxicity. It appears from these experiments, however, that some of the loss in effectiveness of the derris and rotenone sprays can be attributed to the decomposition of the materials in sunlight. There is no satisfactory method at present for preventing the decomposition of these materials.

EFFECT ON ITS EFFECTIVENESS OF ADDING MATERIALS TO DERRIS

A study was made of the effect of adding different materials to derris on its effectiveness as a stomach poison and as a repellent for the Japanese beetle. The results of these experiments are summarized in table 5.

TABLE 5.—*Effect of the addition of other materials to derris on its effectiveness as a stomach-poison insecticide and as a repellent to the Japanese beetle*

Material added to derris	Concentration		Coefficient of effectiveness	Extent of feeding on smartweed foliage	Remarks
	Material	Derris			
	Pounds per 100 gallons	Pounds per 100 gallons			
None	0	2	0	Light	
		4	0	Very light	
		8	0	do	
		16	0	do	
		32	0	do	
Light-pressed fish oil	2	4	0	Light	
		8	0	Very light	
		16	0	do	
		32	0	do	
Molasses	8	0	0	Defoliation	Foliage sticky.
		4	0	Light	
		8	0	Very light	
		16	0	do	
		32	0	do	
	32	0	0	Defoliation	Foliage very sticky.
		4	0	Light	
		8	0	Very light	
		16	0	do	
		32	0	do	
Phenothiazine	2	0	0	Defoliation	Slight narcotic effect on beetles.
		2	0	Moderate	
		4	0	Light	
		8	0	do	
		16	0	Very light	
	4	0	0	Defoliation	Do.
		2	0	Moderate	
		4	0	Light	
		8	0	do	
	8	0	0	Extensive	Definite narcotic effect.
	16	0	0	Moderate	
	32	0	0	do	
Sodium silicate	8	0	0	Defoliation	Derris suspension became red.
		2	0	Moderate	
		4	0	do	
		8	0	Light	
	16	0	0	Defoliation	Do.
		2	0	Extensive	
		4	0	do	
		8	0	Moderate	
Tannic acid	4	0	0	Defoliation	
		4	0	Light	
		8	0	do	
	8	0	0	Defoliation	
		4	0	Very light	
		8	0	do	

It was found that the addition of fish oil to derris did not modify the effectiveness of the material as a stomach poison or as a repellent. When molasses was sprayed on the foliage at the rate of 8 pounds to 100 gallons, the plants were very attractive to the beetles and all the foliage was consumed in 24 hours; when the molasses was used at the rate of 32 pounds to 100 gallons, the beetles were repelled somewhat by the sticky surface of the leaves but defoliated the plants. When derris was mixed with molasses, beetles went readily to the foliage but fed only to a limited extent. Phenothiazine appeared to have a narcotic effect on the beetles, but it was not repellent or toxic. Phenothiazine also appeared to decrease the value of the derris as a repellent.

Sodium silicate solution, containing 6.4 percent Na_2O and 24.7 percent SiO_2, and having a pH of approximately 10.5, when added to derris to increase its adhesiveness, decomposed the derris and destroyed its value as a repellent. Tannic acid, under some conditions in the field, appeared to deter the beetles from feeding. Under these artificial conditions tannic acid was of no value in protecting the foliage from attack. The addition of tannic acid to derris sprays did not appreciably modify their effectiveness.

EFFECT OF VARIOUS STICKERS ON THE REPELLENCY OF DERRIS BEFORE AND AFTER WASHING

It has been observed during the past 2 years that if a rain occurred a few hours after the application of derris or rotenone in the field most of the deposit was removed and the plants were subject to attack by the beetles. It was believed that this mechanical loss by the action of rain, and possibly of wind and dew, was one of the most important factors, if not the most important factor, limiting the effectiveness of derris sprays in this vicinity. The following materials were therefore tried as stickers to increase the adhesiveness of derris to foliage: Fish oil, bone glue, bone glue with triethanolamine, emulsified paraffin, rosin and castor oil, castor oil and glue emulsified with oleic acid and triethanolamine, and tannic acid. The fish oil and tannic acid were added directly to the derris; the paraffin, with a melting point of about 110° F., was emulsified with oleic acid and triethanolamine according to the procedure outlined by Ginsburg (*12*); the rosin and castor oil were heated on a water bath until the rosin was in solution; the mixture of castor oil, glue, oleic acid, and triethanolamine was added to an equal weight of water and boiled until homogeneous.

These materials were added in different proportions to the derris suspensions and sprayed on smartweed plants. After the spray deposit had dried, half of the sprayed plants were subjected for 10 minutes to a coarse spray of water under 20 pounds' pressure. When the plants were dry, they were placed in cages with 200 beetles under the controlled conditions previously described. The effect of the different stickers on derris, before and after washing, is given in table 6.

It was apparent that derris without a sticker is readily removed from foliage by washing, as beetles that refused to feed on the unwashed foliage consumed all the foliage after it had been washed. The addition of fish oil, bone glue, and tannic acid did not appear to increase the adhesiveness of the deposit. When triethanolamine was

TABLE 6.—*Effect of various stickers on derris, used at the rate of 4 pounds to 100 gallons, as a repellent to the Japanese beetle, before and after washing the foliage with water*

Stickers added to derris	Concentration of stickers [1]	Extent of feeding on smartweed foliage—	
		Unwashed	Washed
	Pounds per 100 gallons		
None	0	Very light	Defoliation.
Light-pressed fish oil	2	do	Do.
Bone glue	4	do	Do.
	8	do	Extensive.
Bone glue and triethanolamine	2–2	Moderate	Defoliation.
	4–4	Extensive	Do.
	8–8	do	Do.
Rosin and castor oil	1–1	Light	Extensive.
	2–2	do	Do.
	4–4	do	Moderate.
Castor oil, oleic acid, bone glue, and triethanolamine	1.27–0.12–0.30–0.30	do	Do.
	1.38–0.12–0.20–0.30	do	Do.
	1.48–0.12–0.10–0.30	do	Do.
Paraffin, oleic acid, and triethanolamine	0.80–0.08–0.03	do	Extensive.
	4–0.40–0.15	do	Moderate.
	8–0.8–0.3	do	Do.
	16–1.6–0.6	do	Do.
	40–4–1.6	do	Light.
Tannic acid	4	do	Extensive.
	8	do	Do.

[1] Where combination stickers are used, concentrations are given in order named.

added to the derris and bone-glue mixture, the extent of feeding on unwashed foliage increased with increments of triethanolamine, indicating that the addition of this material was decomposing the derris.

The addition of rosin and castor oil, or of castor oil, oleic acid, bone glue, and triethanolamine, to the derris suspensions did not seem to decompose the derris and appeared to reduce the loss of the material from the foliage during the washing operation. When paraffin was emulsified and used at the concentration of 40 pounds to 100 gallons, the loss of derris appeared to be prevented. In this case the leaves were entirely covered with a waxy deposit, which was resistant to water. The use of paraffin in this concentration is impractical and is likely to injure the foliage. When smaller quantities of paraffin were added to the derris, it was apparent that some of the material was removed by the washing.

It has been demonstrated that derris is readily removed from the foliage by washing, and that some sticker is needed to make the spray satisfactory in the field. However, the results with the materials tested during the summer of 1934 showed that none of these materials was satisfactory and indicate the necessity for further experimentation to develop a suitable sticking agent for derris.

SUMMARY

A study was made of derris and its components under artificially controlled conditions to obtain further information on the value of these materials for the control of the Japanese beetle.

Rotenone was found to be 2.3 times as effective as neutral potassium oleate as a contact insecticide, deguelin was equally as effective as the soap, and derris was 0.387 times as effective. When an equal weight of castor oil was added to the derris, the coefficient of effectiveness was increased to 0.556. When added to a 0.125-percent solution

of coconut-oil soap, which alone did not kill beetles, the effectiveness of derris was increased to 1.765 times that of potassium oleate. These data indicate that if a small quantity of soap is added to the derris spray in the field the effectiveness of the material as a contact insecticide may be greatly increased.

Derris and its components, with the possible exception of rotenone, were found to be of little value as stomach-poison insecticides against this insect. Rotenone when used at the rate of 4 pounds to 100 gallons was 53.4 percent as effective as acid lead arsenate used at the rate of 8 pounds to 100 gallons of water.

Beetles fed only slightly on derris when used at the rate of 2 pounds to 100 gallons. It is apparent that the repellent action of derris can be attributed to its rotenone and deguelin content. Toxicarol, tephrosin, and the derris resin from which all crystalline substances had been removed had little deterrent effect on the insect.

Under artificial conditions of heavy infestation, where the beetles were stimulated to feed and had no choice between sprayed and unsprayed foliage, derris and rotenone afforded complete protection to the fruit and foliage of Yellow Transparent apples, being superior to acid lead arsenate and the lime sprays, which have given satisfactory results under favorable field conditions.

Exposure to ultraviolet light definitely reduced the value of rotenone as a stomach poison against the Japanese beetle. It was not possible to detect the change in effectiveness of derris because of the low toxic value of the material. The value of derris and rotenone as repellents was only slightly reduced by exposure to ultraviolet light for 24 hours, indicating that the repellent effect is not destroyed so rapidly as the toxicity.

Fish oil and tannic acid did not modify the effectiveness of derris as a stomach-poison insecticide or as a repellent. Sodium silicate decomposed the derris and destroyed its value as a repellent.

Derris is readily washed from foliage by water. It is believed that this mechanical loss by the action of rain, and possibly dew, is one of the most important factors limiting the effectiveness of derris sprays for control of the Japanese beetle. Several stickers were tested, but none of them proved satisfactory.

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THE AMMONIATION OF WASTE SULPHITE LIQUOR AND ITS POSSIBLE UTILIZATION AS A FERTILIZER MATERIAL [1]

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INTRODUCTION

Waste sulphite liquor is a byproduct or waste material produced in connection with the preparation of wood pulp by the sulphite process. When wood and similar lignified materials are heated with a solution of sulphurous acid and acid sulphites, the lignin is converted into water-soluble lignin sulphonic acids, leaving the cellulose in a more or less pure condition. The solution containing the lignin sulphonic acids, various carbohydrates, organic acids, and excess of sulphurous acid and bisulphites is generally discarded. In this country alone, approximately 1,500,000 tons of lignin are annually discharged from the various mills producing wood pulp.

Waste sulphite liquor has been the subject of numerous investigations, and there is at present an extensive chemical and patent literature dealing with the utilization or possible utilization of this industrial waste material. As yet, however, no method has been developed for the economic utilization of waste sulphite liquor, although attempts have been made to use it in the preparation of dyes, adhesives, and binding, sizing, and tanning materials, and as a fertilizer.

It is now believed by many agricultural chemists that humus or soil organic matter, which greatly improves the texture and moisture-holding capacity of the soil, has been derived to a considerable extent from lignin. The fact that even under the most favorable conditions, the rate of decomposition of lignin by soil micro-organisms is much less than that of the other major plant constituents, has been advanced as an argument in favor of the view that much of the soil humus was formed from lignin. Lignin constitutes by far the largest percentage of the organic matter of waste sulphite liquor. In view of the fact that there is at present a demand for nitrogenous organic fertilizer materials, largely because the nitrogen contained therein becomes available to the plant gradually, it seemed worth while to attempt to introduce nitrogen into lignin and possibly into the other organic components of the waste sulphite liquor and thus obtain a nitrogenous product having the nitrogen chemically bound to the organic material. The results of a preliminary investigation of the ammoniation of waste sulphite liquor were presented in a previous publication (9).[2] In the present paper results are presented on the ammoniation of waste sulphite liquor under various experimental conditions and on the composition of the products thus obtained,

together with some data on the ammonification and nitrification of these products by soil micro-organisms and their evaluation as fertilizer materials by vegetative greenhouse experiments.

REVIEW OF LITERATURE

Because of its relatively high content of sulphur and organic matter, various attempts have been made to utilize waste sulphite liquor as a fertilizer either by itself or in combination with other materials rich in phosphorus, potash, or nitrogen. The percentage of phosphorus, potash, and nitrogen in sulphite liquor itself is practically nil. A review of the older literature on the utilization of waste sulphite liquor as a fertilizer material has been given by Johnsen and Hovey (*7*), and need not be repeated here. Among the more recent publications on the utilization of waste sulphite liquor as a fertilizer may be mentioned those of Bokorny (*2, 3*) and Görbing (*5*). Bokorny applied to the soil some neutralized waste sulphite liquor from which the sugars had been removed by fermentation with yeast, which had then been concentrated to one-fifth of its original volume, and obtained increased yields of barley, beans, peas, wheat, and potatoes. Görbing, however, failed to confirm the findings of Bokorny.

Feustel and Byers (*4*) heated lignin from corncobs, and also waste sulphite liquor, with aqueous ammonia for 3 hours at 150° C. The percentage of nitrogen combined with the lignin and waste sulphite liquor amounted to 2.37 and 5.06 percent, respectively.

Scholl and Davis (*11*) heated the residue from waste sulphite liquor for 24 hours with liquid ammonia at 125° C. and obtained a product which contained 4.58 percent of nitrogen. No data on the availability of the nitrogen in the ammoniated product as determined by chemical methods and by greenhouse experiments were presented.

Pinck, Howard, and Hilbert (*10*), by treating alkali lignin from corncobs with liquid ammonia for 24 hours at 180° C., obtained a product which contained 7 percent of nitrogen. They determined the various groups of nitrogenous complexes in their product but presented no data on the availability to plants of the nitrogen in their ammoniated lignin.

MATERIAL AND APPARATUS

All the ammoniation experiments described in this paper were conducted on a batch of sulphite liquor which had been obtained from a paper mill.[3] A portion of this was evaporated to dryness, dried at 105° C., and analyzed by the standard methods of the Association of Official Agricultural Chemists (*1*). The following results were obtained: Total P_2O_5, none; total N, 0.04 percent; total K_2O, 0.15 percent.

For the first group of experiments, a portion of the waste sulphite liquor was neutralized with ammonia, evaporated to dryness, and dried at 105° C. The total nitrogen content of this as determined by the Kjeldahl-Gunning-Arnold method (*1*) amounted to 2.6 percent.

The second group was conducted with material prepared as follows: Ammonia gas was passed into the sulphite liquor until the desired

[3] The sulphite liquor used in these experiments was kindly supplied by Brown Co., of Berlin, N. H.

concentration of ammonia was obtained, and this solution was then heated in the autoclave. Each solution thus prepared was analyzed for total NH_3 and free NH_3. The former was determined by accurately weighing out in a weighing bottle 4 to 5 g of the solution, and making up to volume in a volumetric flask. An aliquot of this was measured into a Kjeldahl flask, 200 cc of distilled water and 10 cc of 50-percent sodium hydroxide were added, and this solution was distilled into a definite volume of a standard acid solution. The free ammonia was determined in a similar manner, except that no sodium hydroxide solution was added to the Kjeldahl flask containing the solution to be analyzed.

The apparatus used in all the ammoniation experiments consisted of two steel bombs of approximately 500-cc capacity. These bombs were fastened to a carrier and revolved in an oil bath provided with thermostatic control. A full description of the apparatus has been given elsewhere (*6*, *8*).

EXPERIMENTAL PROCEDURE

The experimental procedure for the first group of experiments was as follows: Into each bomb 50 g of the dry material, which had been neutralized with ammonia, and the requisite quantity of aqueous ammonia (28 percent NH_3) were placed. The bombs were rotated in the oil bath maintained at a definite temperature for a given period. In each experiment, the reaction products from the two bombs were combined, evaporated to dryness on the steam bath, and dried at 105° C. The product was analyzed for total nitrogen, ammoniacal nitrogen, water-soluble organic nitrogen, and water-insoluble organic nitrogen soluble in neutral permanganate. The analytical methods of the Association of Official Agricultural Chemists (*1*) were employed.[4]

For the second group of experiments 300 g of the sulphite liquor, containing the desired concentration of ammonia, was placed in each bomb and heated for a definite period in the oil bath maintained at a definite temperature. The reaction products from the two bombs were combined, evaporated to dryness on the steam bath, and dried at 105° C. The products obtained were analyzed for the various forms of nitrogen, as already described for the first group.

RESULTS

AMMONIATION OF WASTE SULPHITE LIQUOR AND ITS NEUTRALIZED DRY RESIDUE

Table 1 shows that at a temperature of 200° C. and at a ratio of 600 cc of aqueous ammonia (28 percent NH_3) to 100 g of the dry neutralized residue from waste sulphite liquor the percentage of total nitrogen increased with increase in the period of heating, whereas the percentage of ammoniacal nitrogen decreased. The percentage of water-soluble organic nitrogen expressed as percentage of the total nitrogen decreased as the heating period increased. The percentage of nitrogen of the water-insoluble organic nitrogen soluble in neutral permanganate was somewhat irregular, ranging from 83.6 to 91.3 percent.

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[4] The samples from the ammoniation experiments 5, 11, 12, and 18 were analyzed in the laboratory of the Division of Soil Fertility Investigations, Bureau of Plant Industry, under the supervision of E. C. Shorey for ammoniacal nitrogen, water-soluble organic nitrogen, and water-insoluble organic nitrogen soluble in neutral permanganate solution.

TABLE 1.—*Analysis of dry product after ammoniation of solids of waste sulphite liquor with 28-percent aqueous ammonia at 200° and at 220° C., with various periods of heating*

[100 g of dry neutralized residue of waste sulphite liquor and 600 cc of aqueous ammonia (28 percent NH_3) were used for each experiment]

200° C.

Experiment no.	Time of heating	Total nitrogen	Ammoniacal nitrogen	Water-soluble organic nitrogen		Water-insoluble organic nitrogen			
				In sample	Of total nitrogen	In sample	Of total nitrogen	Soluble in neutral permanganate	
								In sample	Of water-insoluble nitrogen
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	4	7.30	1.23	6.38	87.4	0.92	12.6	0.82	89.1
2	8	8.32	.36	3.32	39.9	5.00	60.1	4.28	85.6
3	12	8.83	.20	3.32	37.6	5.51	62.4	4.61	83.6
4	16	8.56	.22	3.08	36.0	5.48	64.0	4.76	86.9
5	20	9.07	.19	2.86	31.5	6.21	68.5	5.67	91.3

220° C.

6	4	8.82	0.33	3.04	34.5	5.78	65.5	5.01	86.7
7	8	9.56	.20	3.52	36.8	6.04	63.2	5.22	86.4
8	12	9.95	.13	3.83	38.5	6.12	61.5	5.31	86.8
9	16	9.92	.15	3.68	37.1	6.24	62.9	5.38	86.2
10	20	10.55	.09	3.65	34.6	6.90	65.4	5.59	81.0
11	30	10.14	.05	3.44	33.9	6.70	66.1	4.16	62.1

When the ammoniation was carried out at a higher temperature, namely, at 220° C., the percentage of total nitrogen in the product increased as the heating period increased, reaching a maximum at a heating period of 20 hours, after which it declined slightly. For the most part, the percentage of ammoniacal nitrogen decreased in a regular manner as the heating period increased. The percentage of water-soluble organic nitrogen expressed as percentage of the total nitrogen showed very little variation with increase in the heating period. The percentage of nitrogen soluble in neutral permanganate expressed as percentage of the water-insoluble nitrogen was affected only to a slight extent when the heating period ranged from 4 to 16 hours. However, the products obtained by heating for 20 to 30 hours did show substantial decreases in the percentage of water-insoluble nitrogen soluble in neutral permanganate.

It will be observed that at 220° C. the ammoniation proceeded much more rapidly than at 200°. At the temperature of 220° and at a heating period of only 4 hours a product was obtained which contained the same percentage of total nitrogen as the material from experiment 3, where the heating period was 12 hours. With the exception of experiment 1, the percentages of water-soluble organic nitrogen in the products from the two series of experiments were of the same general order of magnitude. In the series of experiments conducted at 220° the percentage of water-insoluble nitrogen soluble in neutral permanganate decreased substantially when the reaction mixture was heated for 20 or 30 hours.

In table 2 are presented results of two series of experiments which were conducted for the purpose of ascertaining the optimum ratio of

ammonia to the neutralized sulphite liquor residue. The experiments were carried out at 200° and 220° C., and the optimum reaction period for total nitrogen previously determined, namely, 20 hours (table 1), was employed.

TABLE 2.—*Analysis of dry product after ammoniation of solids of waste sulphite liquor with various quantities of 28-percent aqueous ammonia, at 200° and at 220° C.*

[100 g of dry neutralized residue of waste sulphite liquor were used for each experiment. Time of heating was 20 hours]

200° C.

Experiment no.	Aqueous ammonia added	Total nitrogen	Ammoniacal nitrogen	Water-soluble organic nitrogen		Water-insoluble organic nitrogen			
				In sample	Of total nitrogen	In sample	Of total nitrogen	Soluble in neutral permanganate	
								In sample	Of water-insoluble nitrogen
	Cc	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
12	500	9.09	0.12	3.69	40.6	5.40	59.4	4.16	77.0
13	400	8.96	.33	3.15	35.2	5.81	64.8	5.02	86.4
14	300	8.67	.24	2.82	32.5	5.85	67.5	4.83	82.6
15	200	8.51	.11	2.39	28.1	6.12	71.9	4.22	68.9
16	100	7.92	.06	1.81	22.9	6.11	77.1	4.12	67.4

220° C.

Experiment no.	Aqueous ammonia added	Total nitrogen	Ammoniacal nitrogen	In sample	Of total nitrogen	In sample	Of total nitrogen	In sample	Of water-insoluble nitrogen
17	500	9.88	0.07	3.09	31.3	6.79	68.7	5.22	76.9
18	400	10.00	.09	3.07	30.7	6.93	69.3	4.93	71.1
19	300	9.83	.11	3.23	32.9	6.60	67.1	4.32	65.5
20	200	9.53	.07	2.57	27.0	6.96	73.0	3.90	56.0
21	100	8.47	.05	1.48	17.5	6.99	82.5	3.33	47.6

Table 2 shows that at 200° C. and with a ratio of only 400 to 500 cc of aqueous ammonia to 100 g of neutralized sulphite liquor residue, an ammoniated product was obtained which contained practically the same percentage of total nitrogen as when 600 cc of 28-percent ammonia was used (experiment 5, table 1). The percentage of water-soluble organic nitrogen in the product decreased with the decrease of the ratio of aqueous ammonia to the dry residue of waste sulphite liquor. The nitrogen soluble in neutral permanganate, expressed as percentage of the water-insoluble nitrogen, declined considerably when the ratio of aqueous ammonia to the neutralized residue of waste sulphite liquor was 1:1 or 2:1.

In the experiments conducted at 220° C. the percentage of total nitrogen in the product decreased with the decrease in the ratio of ammonia to the sulphite liquor residue. When 600 cc of aqueous ammonia was used the percentage of total nitrogen in the product amounted to 10.55 (experiment 10, table 1), while when only 100 cc was used the percentage of nitrogen was 8.47. The percentage of water-soluble organic nitrogen as well as the percentage of water-insoluble nitrogen soluble in neutral permanganate decreased decidedly when lower ratios of ammonia with respect to the residue of waste sulphite liquor were used.

In table 3 and in the following tables data are presented on the direct ammoniation of waste sulphite liquor. Instead of ammoniating the neutralized residue of waste sulphite liquor, ammonia gas

was passed into the raw liquor itself until the desired concentration of ammonia was attained, and the solution thus obtained was then heated under pressure in an autoclave under definite conditions of temperature and time of heating.

TABLE 3.—*Analysis of dry product after ammoniation of waste sulphite liquor with ammonia gas at various temperatures and with various periods of heating*

[300 g of sulphite liquor was used]

220° C.

Experiment no.	Time of heating	Concentration of ammonia		Total nitrogen	Ammoniacal nitrogen	Water-soluble organic nitrogen		Water-insoluble organic nitrogen		Soluble in neutral permanganate	
		Total	Free			In sample	Of total	In sample	Of total	In sample	Of water-insoluble nitrogen
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
22	10	12.0	11.7	9.42	0.11	2.55	27.1	6.87	72.9	6.10	88.8
23	15	12.0	11.7	9.77	.12	2.49	25.5	7.28	74.5	6.23	85.6
24	20	12.0	11.7	9.30	.14	2.43	26.1	6.87	73.9	5.59	81.4
25	25	12.0	11.7	9.96	.10	2.65	26.6	7.31	73.4	6.19	84.7
26	10	14.19	14.5	9.96	.18	2.19	22.0	7.77	78.0	6.14	79.0
27	15	14.9	14.5	9.70	.10	2.30	23.7	7.40	76.3	5.89	79.6
28	20	14.9	14.5	10.15	.08	1.82	17.9	8.33	82.1	6.82	81.9
29	25	14.9	14.5	10.27	.10	1.99	19.4	8.28	80.6	6.79	82.0
30	10	27.0	26.6	10.18	.08	2.94	28.9	7.24	71.1	6.05	83.6
31	15	27.0	26.6	10.43	.07	3.33	31.9	7.10	68.1	5.92	83.4
32	20	27.0	26.6	11.15	.07	3.19	28.6	7.96	71.4	6.59	82.8
33	25	27.0	26.6	10.58	.04	2.86	27.0	7.72	73.0	6.26	81.1

200° C.

34	10	11.4	11.1	7.70	0.56	3.20	41.6	4.50	58.4	3.89	86.4
35	15	11.4	11.1	8.21	.40	2.66	32.4	5.55	67.6	4.78	86.1
36	20	11.4	11.1	8.21	.41	2.57	31.3	5.64	68.7	4.99	88.5
37	25	11.4	11.1	8.49	.17	2.45	28.9	6.04	71.1	4.40	72.8
38	10	14.6	14.2	8.46	.59	2.78	32.9	5.68	67.1	5.03	88.6
39	15	14.6	14.2	8.72	.28	2.47	28.3	6.25	71.7	5.39	86.2
40	20	14.6	14.2	9.67	.13	2.71	28.0	6.96	72.0	6.19	88.9
41	25	14.6	14.2	9.15	.18	2.72	29.7	6.43	70.3	5.52	85.8
42	10	17.9	17.8	7.78	.62	3.20	41.1	4.58	58.9	4.01	87.6
43	15	17.9	17.8	7.92	.61	2.64	33.3	5.28	66.7	4.25	80.5
44	20	17.9	17.8	8.43	.43	2.78	33.0	5.65	67.0	4.31	76.3
45	25	17.9	17.8	8.53	.35	2.80	32.8	5.73	67.2	4.39	76.6
46	10	25.6	25.4	8.98	.48	3.05	34.0	5.93	66.0	5.09	85.8
47	15	25.6	25.4	9.33	.32	3.17	34.0	6.16	66.0	5.08	82.5
48	20	25.6	25.4	9.47	.38	3.51	37.1	5.96	62.9	4.97	83.4
49	25	25.6	25.4	9.13	.19	2.78	30.4	6.35	69.6	5.67	89.3

180° C.

50	10	11.4	11.1	7.00	1.60	5.63	80.4	1.37	19.6	1.28	93.4
51	15	11.4	11.1	7.51	1.34	4.60	61.3	2.91	38.7	2.48	85.2
52	20	11.4	11.1	7.38	.86	3.29	44.6	4.09	55.4	3.60	88.0
53	25	11.4	11.1	7.49	.82	3.56	47.5	3.93	52.5	3.29	83.7
54	10	14.6	14.2	6.45	1.87	5.75	89.1	.70	10.9	.65	92.9
55	15	14.6	14.2	7.41	1.55	5.62	75.8	1.79	24.2	1.71	95.5
56	20	14.6	14.2	7.27	1.48	4.53	62.3	2.74	37.7	2.45	89.4
57	25	14.6	14.2	7.76	1.43	4.56	58.8	3.20	41.2	2.82	88.1
58	10	17.9	17.8	7.33	1.92	6.34	86.5	.99	13.5	.93	93.9
59	15	17.9	17.8	7.10	1.70	5.28	74.4	1.82	25.6	1.68	92.3
60	20	17.9	17.8	7.29	1.69	5.07	69.5	2.22	30.5	1.94	87.4
61	25	17.9	17.8	7.82	1.40	4.52	57.8	3.30	42.2	3.17	96.1
62	10	25.6	25.4	7.25	.55	4.29	59.2	2.96	40.8	2.70	91.2
63	15	25.6	25.4	7.62	1.14	4.53	59.4	3.09	40.6	2.92	94.5
64	20	25.6	25.4	8.00	.92	3.82	47.7	4.18	52.3	3.76	89.9
65	25	25.6	25.4	8.66	.70	3.57	41.2	5.09	58.8	4.49	88.2

In table 3 data are given on the ammoniation at 220°, 200°, and 180° C. In the experiments conducted at 220° the concentrations of ammonia were 12.0, 14.9, and 27.0 percent. The reaction periods ranged from 10 to 25 hours. The data show that the percentage of total nitrogen in the product was only slightly affected by the variation of the concentration of ammonia from 12.0 to 27.0 percent. In each of the three series of experiments the increase in the percentage of total nitrogen was not especially pronounced when the reaction period was increased from 10 to 25 hours. The ammoniacal nitrogen in the product was very little affected by either the change in concentration of ammonia or the variation of the reaction period. The percentages of water-soluble organic nitrogen and of water-insoluble organic nitrogen soluble in neutral permanganate solution were rather irregular in all the three series of experiments. The percentage of water-insoluble organic nitrogen soluble in neutral permanganate solution, expressed as percent of the total water-insoluble organic nitrogen, was generally 80 percent or above.

In the series of experiments conducted at 200° C. the reaction periods also ranged from 10 to 25 hours, and the concentrations of ammonia were 11.4, 14.6, 17.9, and 25.6 percent. As a rule, with the same heating period, the total nitrogen in the product increased with the increase in the concentration of ammonia in the sulphite liquor. However, in experiments 42, 43, 44, and 45, where the concentration of ammonia in the sulphite liquor was 17.9 percent, the percentage of total nitrogen in the product, for some unknown reason, did not show a corresponding increase. Nearly all the experiments recorded in table 3 showed an increase in the percentage of total nitrogen as the period of heating was increased. In practically all the experiments, the ammoniacal nitrogen in the product decreased with the increase in the time of reaction.

In practically all these experiments the percentage of water-soluble organic nitrogen in the ammoniated product as well as the percentage of the water-insoluble nitrogen soluble in neutral permanganate showed a downward trend with the increase in the reaction period from 10 to 25 hours. In the main, the results obtained in experiments 38, 39, 40, and 41, where the concentration of ammonia in the sulphite liquor amounted to 14.6 percent, compare rather favorably as to the percentage of total nitrogen in the ammoniated product and the percentage of water-insoluble organic nitrogen soluble in neutral potassium permanganate solution with those experiments in which sulphite liquor containing higher concentrations of ammonia were employed.

In the experiments conducted at 180° C. the same concentrations of ammonia were used as in the experiments conducted at 200°. The heating periods also ranged from 10 to 25 hours. In practically all these experiments the percentage of total nitrogen in the ammoniated product increased with the increase in the reaction period. However, in the case of the ammoniacal nitrogen in the reaction product, this relationship was reversed, that is, the longer the reaction period the smaller the percentage of ammoniacal nitrogen. This was found to hold also in the experiments recorded in the first two sections of the table. At a temperature of 180° the concentration of ammonia in the sulphite liquor ranged from 11.1 to 25.4 percent without greatly affecting the percentage of total nitrogen in the product. In all

experiments the percentage of water-soluble organic nitrogen decreased steadily with the increase in the reaction period. The percentage of water-insoluble nitrogen soluble in neutral permanganate solution showed a similar tendency, although the results were not so regular and consistent.

AMMONIFICATION AND NITRIFICATION OF NEUTRALIZED AND AMMONIATED RESIDUES OF WASTE SULPHITE LIQUOR

The following experiments [5] were carried out to determine the rate of ammonification and nitrification of the neutralized [6] and ammoniated residues of waste sulphite liquor by soil micro-organisms.

The first series of experiments was conducted for the purpose of ascertaining the relative rates of ammonification of three different preparations of ammoniated waste sulphite liquor [7] as compared with cottonseed meal and casein. For every experiment 25 g of soil in 100 cc of tap water was mixed with a quantity of test substance equivalent to 60 mg of nitrogen, and the mixtures were allowed to incubate for 7 days. The results showed that whereas casein and cottonseed meal were ammonified to the extent of 72 and 18 percent, respectively, the three preparations of ammoniated waste sulphite liquor were ammonified only to the extent of 7, 6, and 5 percent, respectively.

In the second series of experiments the rate of nitrification of a preparation of ammoniated waste sulphite liquor [8] was investigated. The effect of the dry residue of waste sulphite liquor itself, as well as the neutralized residue of waste sulphite liquor, on the nitrification of ammonium sulphate was studied. For each experiment 100-g portions of Keyport soil containing 2 g of calcium carbonate were used, and the mixture was incubated for 1 month under optimum moisture conditions. For the control experiment a quantity of ammonium sulphate equivalent to 30 mg of nitrogen was taken. In other experiments 0.2-, 0.6-, and 1.0-g quantities, respectively, of the dry residue of waste sulphite liquor were employed in addition to the same quantities of ammonium sulphate as was used in the control. The results show that whereas in the control experiment the ammonium sulphate was nitrified to the extent of 84.7 percent, in the experiments where 0.2-, 0.6-, and 1.0-g quantities of the dry residue of waste sulphite liquor had been added, nitrification had proceeded only to the extent of 75.7, 64, and 51.3 percent, respectively, thus indicating that the residue of waste sulphite liquor is definitely toxic to the nitrifying bacteria. When the neutralized residue of waste sulphite liquor was used in place of the unneutralized material, no such toxic effect on the nitrifying bacteria was noted. The ammoniated residue of waste sulphite liquor nitrified in the experimental period of 1 month only to the extent of approximately 20 percent, thus indicating that the nitrogen in this material is rather firmly bound with the organic matter of the waste sulphite liquor. Presumably, upon the destruction of the organic matter the residual nitrogen would become available.

In table 4 data are presented on the relative rates of nitrification of the ammoniated residue of waste sulphite liquor, ammonium sulphate, and blood meal.

[5] All experiments on the ammonification and nitrification by soil micro-organisms recorded in this paper were carried out by N. R. Smith of the Division of Soil Microbiology, Bureau of Plant Industry.
[6] Waste sulphite liquor neutralized with ammonia, evaporated to dryness, and dried at 105° C.
[7] Products from experiments 2, 4, and 10, table 1.
[8] Product from experiment 32, table 3.

TABLE 4.—*Nitrate nitrogen produced by nitrification of ammonium sulphate, blood meal and ammoniated residue of waste sulphite liquor*

SANDY LOAM FROM ARLINGTON EXPERIMENT FARM, ROSSLYN, VA.

Experiment no.	Substance	Nitrate nitrogen found when incubated for—					
		30 days		60 days		90 days	
		Quantity	Percentage of total nitrogen	Quantity	Percentage of total nitrogen	Quantity	Percentage of total nitrogen
		Milligrams	*Percent*	*Milligrams*	*Percent*	*Milligrams*	*Percent*
1	Ammonium sulphate	24.8	82.7	25.2	84.0	23.4	78.0
2	Blood meal	15.2	50.7	15.3	51.0	12.8	42.7
3	Ammoniated residue of waste sulphite liquor.[1]	6.3	21.0	7.0	23.3	6.0	20.0
4	___do.[2]	4.7	15.7	5.6	18.7	5.2	17.3
5	___do.[3]	4.5	15.0	4.5	15.0	4.8	16.0

KEYPORT CLAY LOAM FROM WALKER HILL, VA.

6	Ammonium sulphate	25.0	83.3	25.3	84.3	24.3	81.0
7	Blood meal	12.6	42.0	14.1	47.0	15.2	50.7
8	Ammoniated residue of waste sulphite liquor [1]	5.8	19.3	6.3	21.0	6.0	20.0
9	___do.[2]	3.9	13.0	4.6	15.3	5.0	16.7
10	___do.[3]	4.7	15.7	4.4	14.7	4.0	13.3

[1] Product from experiment 32, table 3.
[2] Product from experiment 4, table 1.
[3] Product from experiment 6, table 1.

For each experiment 2 g of calcium carbonate and the equivalent of 30 mg of nitrogen were added to 100 g of sifted soil and incubated at 28° C. under optimum moisture conditions. All experiments were carried out in triplicate, and the figures in table 4 in each case represent the mean of three values. In every case a correction was applied for the nitrate nitrogen in the soil.

It will be observed from table 4 that all three preparations of ammoniated waste sulphite liquor nitrified much more slowly than either blood meal or ammonium sulphate. Apparently, in all the materials tested, most of the nitrification occurred in the first 30 days. Blood meal, however, was nitrified from two to three times as rapidly as the three samples of ammoniated waste sulphite liquor.

EVALUATION OF AMMONIATED WASTE SULPHITE LIQUOR AS A PLANT FOOD

A waste material that can be treated so that it will contain approximately 11 percent of nitrogen deserves consideration as a possible plant food material. In view of the fact that the ammoniated waste sulphite liquor is practically free of phosphoric acid (P_2O_5) and the percentage of potash (K_2O) is negligible, the material, so far as being a potential plant food is concerned, is to be valued merely as a source of nitrogen. With a fairly high nitrogen content such a product may possess value for fertilizer or soil-improvement purposes.

While it is true that the nitrogen content of the dry residue of ammoniated waste sulphite liquor is exceeded by a number of inorganic nitrogen materials, such as sodium nitrate and ammonium sulphate, and by certain organic compounds used as fertilizer mate-

rials, including urea and cyanamid, it does compare rather favorably with certain organic nitrogen fertilizer materials, such as castor pomace, with an average nitrogen content of about 5 percent; cottonseed meal, with approximately 7 percent of nitrogen; fish scrap, with 7 to 10 percent of nitrogen; tankage, with a nitrogen content of 5 to 10 percent; and dried blood, with a percentage of nitrogen ranging from 9 to 14.

In order to determine the availability of the nitrogen in the ammoniated residue of waste sulphite liquor to plants, as compared with certain standard nitrogen fertilizer materials, vegetative pot tests were conducted under greenhouse conditions at the Arlington Experiment Farm, Rosslyn, Va. Three nitrogen fertilizer materials were selected for comparative purposes, namely, high-grade dried blood, sodium nitrate, and ammonium sulphate. The tests were conducted in 1-gallon glazed pots capable of holding 5 kg of soil. Norfolk loamy fine sand with a pH of 4.8 was used in the first tests. All tests involving any particular fertilizer treatment were made in triplicate unless otherwise stated. In subsequent tests, a similar soil was used, except that the pH was 5.8 instead of 4.8. Millet was used as the indicator crop in all tests. In the tables, the weights of millet obtained are recorded on a moisture-free basis.

In the first series of pot experiments, ammoniated materials ranging in nitrogen content from 2.60 to 9.07 percent were compared with dried blood and a mixture of sodium nitrate and ammonium sulphate as nitrogen sources. In the sodium nitrate-ammonium sulphate mixture each salt furnished an equal quantity of nitrogen in all the experiments herein reported. The five ammoniated materials were mixed separately with superphosphate (18 percent P_2O_5) and muriate of potash (50 percent K_2O) on a 4-12-4 [9] basis. These five fertilizer mixtures were compared with: (1) A mixture containing only superphosphate and muriate of potash, a 0–12–4 mixture; (2) a 4–12–4 mixture with dried blood as the nitrogen source; and (3) a 4–12–4 mixture with the inorganic salts, sodium nitrate and ammonium sulphate, furnishing the nitrogen. The results of this series of experiments are recorded in table 5.

Table 5 shows that in all vegetative tests a better growth of millet was produced with the ammoniated waste sulphite liquor plus phosphoric acid and potash than with the control, the 0–12–4 mixture. However, in no case was the growth response anywhere near so marked as that resulting from the use of either dried blood or the mixture of inorganic salts as the source of nitrogen in a 4–12–4 mixture.

By giving the 0–12–4 mixture a rating of 100, the mean values of the five series of experiments with the ammoniated residue of waste sulphite liquor, dried blood, and the sodium nitrate-ammonium sulphate mixture are 131, 188, and 196, respectively. The relative order just mentioned reflects the growth response of millet to the different nitrogen sources and clearly indicates a comparatively low availability of the combined nitrogen in the ammoniated materials.

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[9] 4 percent of nitrogen (N), 12 percent of phosphoric acid (P_2O_5), and 4 percent of potash (K_2O).

TABLE 5.—*Yields of millet obtained with ammoniated residue of waste sulphite liquor, with dried blood, and with sodium nitrate—ammonium sulphate mixture as sources of nitrogen*

[In all experiments, except in the control, 4-12-4 fertilizer mixtures were employed. Fertilizer was applied at the rate of 2,000 pounds per acre, which is equivalent to 80 pounds of N, 240 pounds of P_2O_5, and 80 pounds of K_2O. Ten millet plants were grown in each pot. Each weight recorded represents 30 plants (moisture-free basis). The soil reaction was pH 4.8.]

Composition of fertilizer N-P_2O_5-K_2O (percent)	Source of nitrogen in fertilizer mixture	Actual and relative yields on an oven-dry basis [1]					
		Series 1		Series 2		Series 3	
		Actual	Relative	Actual	Relative	Actual	Relative
		Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
0-12-4	No nitrogen (control)	21.6	100	17.1	100	19.1	100
4-12-4	Ammoniated waste sulphite material.	[2]25.7	119	[3]25.7	150	[4]28.3	148
4-12-4	Dried blood	43.9	203	30.5	178	39.6	207
4-12-4	Sodium nitrate-ammonium sulphate.	38.5	178	39.0	228	41.4	216

Composition of fertilizer N-P_2O_5-K_2O (percent)	Source of nitrogen in fertilizer mixture	Actual and relative yields on an oven-dry basis [1]					
		Series 4		Series 5		Average	
		Actual	Relative	Actual	Relative	Actual	Relative
		Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
0-12-4	No nitrogen (control)	25.2	100	27.6	100	22.12	100
4-12-4	Ammoniated waste sulphite material.	[5]29.5	117	[6]33.1	120	28.46	131
4-12-4	Dried blood	42.1	167	50.9	184	41.40	188
4-12-4	Sodium nitrate-ammonium sulphate.	40.0	159	55.	200	42.84	196

[1] Series 1 was planted Dec. 29, 1932, and harvested Mar. 30, 1933 (91 days); series 2 was planted Jan. 25, 1933, and harvested Apr. 13, 1933 (78 days); series 3 was planted Feb. 18, 1933, and harvested Apr. 18, 1933 (59 days); series 4 was planted Mar. 20, 1933, and harvested May 6, 1933 (47 days); series 5 was planted Apr. 27, 1933, and harvested June 6, 1933 (40 days).
[2] For this experiment, the residue obtained by neutralizing some waste sulphite liquor with ammonia and then evaporating to dryness was employed; 2.60 percent of nitrogen.
[3] Product from experiment 1, of table 1 used; 7.30 percent of nitrogen.
[4] Product from experiment 2, of table 1 used; 8.32 percent of nitrogen.
[5] Product from experiment 3, of table 1 used; 8.83 percent of nitrogen.
[6] Product from experiment 5, of table 1 used; 9.07 percent of nitrogen.

In view of the fact that the nitrogen in the ammoniated material is rather firmly bound in some organic combination, it was considered desirable to determine whether the material possessed much, if any, residual fertilizer effect. Accordingly, the pots of soil used in series 3 of table 5 were replanted with millet without any fertilizer re-treatment. The roots of the previous millet crop were screened out before replanting. The actual weights of millet obtained, moisture-free basis, were as follows: No nitrogen, 0-12-4 mixture, 12.15 g; ammoniated residue of waste sulphite liquor [10], 4-12-4 mixture, 9.45 g; dried blood mixture, 7.30 g; and inorganic salts mixture, 5.35 g. Expressed on a relative basis with the 0-12-4 mixture taken as 100, the ammoniated material mixture was 78, the dried blood mixture 60, and the inorganic salts mixture 44, thus apparently indicating that the nitrogen in the ammoniated material was available over a longer period than in either the dried blood or the mixture of inorganic salts. However, the comparatively better showing of the 0-12-4 and the ammo-

[10] Product of experiment 2, table 1, which contained 8.32 percent of nitrogen.

niated material mixtures was more probably due to the effect of greater residues of phosphoric acid and potash left in the soil. As the soil used in these experiments was particularly responsive to phosphoric acid, and to some extent potash, it would seem reasonable to suppose that the influence of the fertilizer mixtures containing nitrogen, especially when in an available form, would be to promote a heavier growth of millet and thereby remove more available phosphoric acid and potash than in those experiments where the no-nitrogen, 0–12–4, fertilizer mixture was used, or where the nitrogen was not readily available as was the case with the ammoniated material. When replanted without additional fertilizer treatment the presumably greater residues of phosphoric acid and potash in the pots of soil where the 0–12–4 and ammoniated material mixtures were originally applied would help to account for the fact that the greater yields of millet plants were produced where the soil had originally received the more readily available nitrogen materials, namely, dried blood and the mixture of inorganic salts.

In order to eliminate as far as possible the probable effect of unequal residues of phosphoric acid and potash, the following pot experiments were carried out: Four samples of ammoniated residue of waste sulphite liquor were used [11] as nitrogen sources in 4–12–4 fertilizer mixtures. As in the previous experiments, dried blood and a mixture of sodium nitrate and ammonium sulphate were employed as standard nitrogen sources. The same type of soil, Norfolk loamy fine sand, was used as in the previous experiments. The pH of the soil was, however, 5.8 instead of 4.8. In these experiments eight pots of soil were used for each fertilizer treatment and termed "original planting." After the millet was harvested four of the eight pots were replanted without re-treatment; the others were re-treated with a mixture of superphosphate and muriate of potash and then replanted. This was done to overcome as far as possible any deficiency of phosphoric acid and potash that might result through the unequal utilization of these fertilizer constituents by the first millet crop. It was felt that in this way a better idea would be obtained regarding any residual effect of the different nitrogen materials. The results obtained are recorded in table 6.

Table 6 shows that insofar as the original planting experiments (columns 3 and 4) are concerned the results in the main confirm those recorded in table 5. While the several preparations of ammoniated material when mixed with compounds furnishing phosphoric acid (P_2O_5) and potash (K_2O) produced a greater growth of millet than the control, 0–12–4 mixture, the standard nitrogen materials, dried blood and the mixture of sodium nitrate and ammonium sulphate, produced even greater yields.

In the two replanted series it will be noted that replanting without the addition of phosphoric acid and potash reduced the yields of millet considerably. However, the yields from the pots to which the ammoniated material had originally been added as a source of nitrogen in a 4–12–4 mixture were in every case greater than those obtained from the pots which had received either dried blood or the sodium nitrate-ammonium sulphate mixture. However, as already pointed out in connection with the experiments recorded in table 5, the better

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[11] Samples 9, 13, and 19 and a preparation made under identical experimental conditions as sample 32 were used. These materials contained 9.92, 8.96, 9.83, and 10.90 percent of nitrogen, respectively.

yields of millet obtained from the control pots, 0–12–4 mixture, and from the pots which had received the ammoniated material as a source of nitrogen were probably due to the effect of greater residues of phosphoric acid and potash.

TABLE 6.—*Yields of millet obtained with ammoniated residue of waste sulphite liquor, with dried blood, and with sodium nitrate-ammonium sulphate mixture as sources of nitrogen in original planting and replanting experiments*

[In all experiments, except in the control, 4–12–4 fertilizer mixtures were employed. Fertilizer was applied at the rate of 2,000 pounds per acre, which is equivalent to 80 pounds of N, 240 pounds of P_2O_5, and 80 pounds of K_2O. Ten millet plants were grown in each pot. All weights recorded represent that of moisture-free material. The soil reaction was pH 5.8.]

Composition of fertilizer $N-P_2O_5-K_2O$ (percent)	Source of nitrogen in fertilizer mixture	Actual and relative yields of millet on an oven-dry basis					
		Original planting (total of 8 pots) [1]		Original planting subdivided and treated [2]			
				Without P_2O_5 and K_2O (4 pots) [3]		With P_2O_5 and K_2O (4 pots) [3]	
		Actual	Relative	Actual	Relative	Actual	Relative
		Grams [4]	*Percent*	*Grams* [5]	*Percent*	*Grams* [5]	*Percent*
0–12–4	No nitrogen (control)	37.15	100.0	4.40	100.0	5.05	100.0
4–12–4	Ammoniated material (9.92 percent N)	53.50	114.0	5.00	113.6	6.35	125.7
4–12–4	Ammoniated material (8.96 percent N)	55.95	150.6	4.00	90.9	6.65	131.7
4–12–4	Ammoniated material (9.83 percent N)	47.6	128.1	4.20	95.4	7.45	147.5
4–12–4	Ammoniated material (10.90 percent N)	58.55	157.6	4.65	105.7	8.60	170.3
4–12–4	Dried blood (13.52 percent N)	71.6	192.7	3.80	86.3	7.30	144.5
4–12–4	Sodium nitrate (high grade)—ammonium sulphate (high grade)	75.2	202.4	2.60	59.1	7.40	146.5

[1] Planted July 26, 1935; harvested Sept. 10, 1935 (46 days).
[2] The "original planting" comprised 8 pots. After the crop of millet was harvested, 4 pots were replanted without adding P_2O_5 and K_2O; the other 4 pots received such treatment, the quantity applied being the same as that applied to the 8 pots in the beginning of the test.
[3] Replanted sets, with and without $P_2O_5+K_2O$; planted to millet on Sept. 16; harvested Oct. 31, 1935 (45 days).
[4] Average weight of 80 millet plants; 10 plants per pot in all experimental work.
[5] Average weight of 40 millet plants; on the basis of 80 millet plants, the calculated weights would of course be doubled.

In columns 7 and 8, table 6, the results of replanting experiments are presented. In these experiments each pot received phosphoric acid and potash but no nitrogen. It was felt that by this treatment the effect of the residual nitrogen fertilizer material could be better determined. The results show that in only one case was there an appreciably greater residual effect from the ammoniated material, namely, the one which contained 10.90 percent of nitrogen.

Figure 1 shows the millet obtained in a rather typical experiment with ammoniated material, dried blood, and the inorganic salt mixture.

In summing up the results of the greenhouse pot tests, comparing ammoniated waste sulphite liquor, dried blood, and a sodium nitrate-ammonium sulphate mixture as sources of nitrogen for millet when grown on Norfolk loamy fine sand, it may be stated definitely that, while the ammoniated material was effective as a source of nitrogen in causing increased yields of millet, in none of the tests conducted did the increases anywhere near equal those recorded for the standard nitrogen sources.

In considering further studies with the ammoniated waste sulphite liquor it would be of interest to obtain data on its effectiveness as a nitrogen source on different crops, on soils of various texture under field conditions and under diverse climatic conditions; its use as a partial rather than as a sole source of nitrogen; its value for physical purposes in fertilizer mixtures; and its adaptability as a soil mulch or for direct incorporation with the soil.

DISCUSSION

Attempts made in the past to use waste sulphite liquor as a fertilizer material (more correctly as a soil conditioner or as a source of soil organic matter, for the untreated liquor itself has practically no fertilizing value) have proved unsuccessful, largely because the liquor is decidedly toxic to plants. This toxicity has generally been
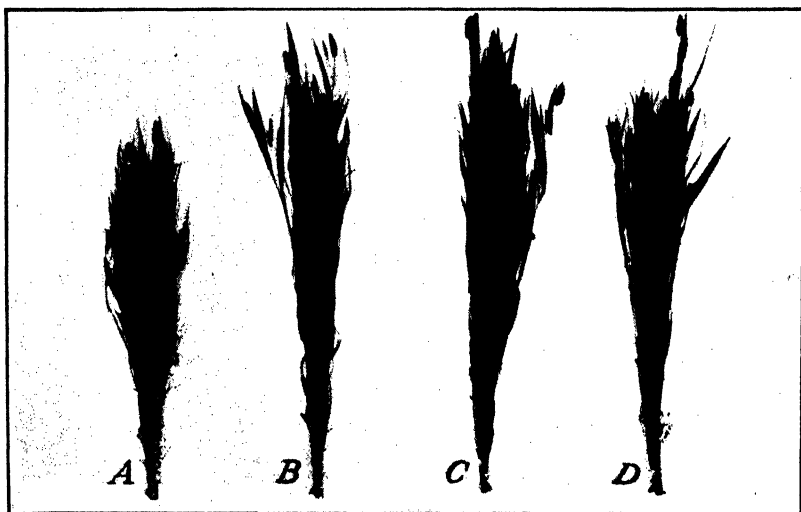
FIGURE 1.—Millet plants (30 in each group) grown in greenhouse pot tests in which different sources of nitrogen were used: *A*, Control (no nitrogen, 0–12–4 mixture); *B*, dried blood; *C*, sodium nitrate-ammonium sulphate; *D*, ammoniated sulphite liquor, 9.92 percent of nitrogen. In *B, C*, and *D*, 4–12–4 fertilizer mixtures were used.

attributed to the sulphurous acid and bisulphites in the liquor. The nitrification experiments recorded in this paper show that even the dry residue of waste sulphite liquor (thus presumably free of sulphurous acid) is toxic to the nitrifying bacteria. However, the various products made by heating waste sulphite liquor, or the neutralized residue of this liquor, with aqueous ammonia under pressure were not toxic either to soil micro-organisms or to millet used as the indicator crop in the vegetative tests.

While the percentage of total nitrogen in the ammoniated products became greater with the increase in the temperature of ammoniation, and within certain limits also with the increase in the reaction period, the availability of the nitrogen, as measured by its solubility in water, decreased. The ammoniated materials were ammonified and nitrified rather slowly by soil micro-organisms, as compared with cottonseed meal and casein and with ammonium sulphate and dried blood,

respectively. While the vegetative greenhouse tests indicated that the ammoniated materials caused increased yields of millet, when grown on Norfolk loamy fine sand, they were not so good in this respect as either dried blood or the mixture of sodium nitrate and ammonium sulphate. The replanting experiments also showed that the nitrogen in the ammoniated material is rather firmly combined. Just how the ammonia combines with the lignin sulphonic acids or with the other components of waste sulphite liquor and in what form the nitrogen occurs in the final product can only be surmised. The behavior of the material would suggest that the nitrogen may be present in some cyclic combination.

Attempts were made to apply the method employed in protein analysis for the determination of the distribution of the various forms of nitrogen in the material, but the results obtained could not be satisfactorily interpreted. Furthermore, it was felt that a method developed for the analysis of pure proteins could not be used in the analysis of such a complex material as the ammoniated residue of waste sulphite liquor. The results did show, however, that in some of the ammoniated products only about 30 percent of the nitrogen was converted into soluble form even after boiling with 20-percent hydrochloric acid for 48 hours.

SUMMARY

A study was made of the ammoniation of waste sulphite liquor with aqueous ammonia, under pressure, at temperatures ranging from 180° to 220° C., and under various conditions of concentration of ammonia and time of reaction. The ammoniations were carried out with the neutralized dry residue of waste sulphite liquor (neutralized with ammonia) and with the waste sulphite liquor itself to which various quantities of ammonia had been added. The results show that under a definite concentration of ammonia in the reaction mixture and for any given reaction period, the percentage of total nitrogen in the product increased with the increase of temperature from 180° to 220°. The percentage of total nitrogen in the product was not greatly affected by the variation, within certain limits, of the concentration of ammonia in the reaction mixture.

When 1 part, by weight, of the neutralized dry residue of waste sulphite liquor, was heated at 220° C. for 20 hours with 6 parts, by volume, of 28-percent aqueous ammonia, a product was obtained which contained 10.55 percent of nitrogen. When ammonia was passed into waste sulphite liquor until the concentration of free ammonia was 14.5 percent, and the resulting solution was heated at 220° for 20 hours, the product obtained contained 10.15 percent of nitrogen.

When the ammoniations were conducted at 200° C. or above, the percentage of ammoniacal nitrogen in the products was generally negligible. The percentage of ammoniacal nitrogen in the products obtained from the ammoniation experiments conducted at 180° ranged from 0.55 to 1.92 percent.

While the percentage of water-insoluble nitrogen showed some variation, it generally increased with the increase in the temperature of ammoniation.

The percentage of insoluble nitrogen soluble in neutral permanganate in the ammoniated product was not greatly affected by the temperature of ammoniation.

The ammoniated products obtained were found to be nontoxic to the micro-organisms of the soil, although their ammonification and nitrification were rather slow as compared with that of casein and cottonseed meal and ammonium sulphate and dried blood, respectively.

Vegetative tests conducted in the greenhouse show that as a source of nitrogen the ammoniated material caused increased yields of millet when grown on Norfolk loamy fine sand, but it was not as efficient in this respect as either dried blood or a mixture of sodium nitrate and ammonium sulphate.

Ammoniated waste sulphite liquor may prove to have value as a potential source of nitrogen, as a soil conditioner, or as a source of humus, but as an immediate source of nitrogen for millet it was found to be considerably less effective than either dried blood or a sodium nitrate-ammonium sulphate mixture.

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A STUDY OF THE EFFECT OF BRUCELLA ABORTUS IN UDDERLESS COWS [1]

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INTRODUCTION

During the early investigations of *Brucella abortus* as the etiological factor of Bang's disease, the organism was thought to be present only in the uterus, fetus, fetal membranes, and vaginal discharge of the aborting cow. Later, however, Schroeder and Cotton (*20*)[2] reported the finding of abortion organisms in the milk of infected cows, and Fabyan (*9*), Seddon (*23*), Cooledge (*5*), and Giltner and his coworkers (*14*) showed conclusively that the udder is a frequent seat of infection.

While the *Brucella abortus* organism has been isolated from tissues of the cow other than those mentioned (*1, 8*), the significance of such localizations in relation to perpetuation of the disease remains in question. Gwatkin (*16*) and others have shown the organism to be present in the placentae and vaginal discharges of infected cows at the time of calving, even though abortion did not occur. According to the reports of Cotton (*6*), Schroeder and Cotton (*21*), Schroeder (*19*), and Fitch et al. (*10*), elimination of *Br. abortus* by way of the vagina usually terminates within 60 days after abortion or calving.

Investigations of Giltner and Bandeen (*13*) and Stafseth (*25*) confirm the observation that the nongravid uterus of the *Brucella* aborted cow is not a persistent location of the infection.

The percentage of cows in a Bang's-diseased herd having udder infection varies. Cooledge (*4*) reports udder infection in 27 percent of the animals under observation, Carpenter (*2*) in 66 percent, Gilman (*12*) in 53.7 percent, Schroeder and Cotton (*22*) in 5 percent, Sheather (*24*) in 34 percent, Fitch and Lubbehusen (*11*) in 9 percent, Carpenter and Baker (*3*) in 56.2 percent, and Graham and Thorp (*15*) in 26.1 percent. It is evident from these data that a high rate of udder infection occurs in some diseased herds.

Cotton (*7*) states that the infection may remain in the udder for at least 6½ years, and that this organ seems to be a reservoir from which the uterus can be reinfected. Other investigations support the observation that the udder may retain the infection over long periods of time.

Mitchell and Duthie (*18*) surgically removed the infected udders and supramammary lymph glands from two reactor cows. Following the operations there was a decline in the agglutinin titer of the cows. One showed a recession from 1:600 to 1:25. The other, however, which had an original titer of 1:2,800, still maintained a titer of 1:100 at the termination of the test, 286 days after the udder had been removed. These investigators suggest caution in drawing conclusions from their experiment, although it would appear that re-

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moval of the infected udders was responsible for the decline in titer which followed the operations.

These references to literature regarding the source and persistence of *Brucella abortus* in affected cows indicate that the udder is the usual seat of localization of the infecting organism in the nonpregnant animal. If the udder is the usual seat of permanent localization of infection in nonpregnant cows, the question arises: Do reactor cows that have aborted and are free of udder infection disseminate *Br. abortus* during subsequent gestations if unbred for 60 to 90 days after aborting and not exposed to other sources of reinfection?

Investigation of this problem requires the use of cows known to be free from udder infection. Diagnosis of udder infection by the use of milk for culture or animal inoculations is not entirely satisfactory as the infected udder may not shed organisms continuously. Similarly, failure to demonstrate *Brucella abortus* agglutinins in the milk is not always a true index of absence of this organism from the udder.

Since available diagnostic methods do not afford unquestionable evidence as to the udder's freedom from *Brucella abortus* infection, an attempt was made in the present experiment to overcome this uncertainty by the use of cows whose udders had been removed surgically.

GENERAL PLAN OF EXPERIMENT

Heifers which showed a negative agglutination reaction to *Brucella abortus*, and whose udders had been removed [3] were raised to sexual maturity in an environment free from *Br. abortus* infection. After reaching maturity they were bred, and when in the fourth to fifth month of gestation they were experimentally exposed to abortion infection. After aborting, these cows were rebred and held under observation until the termination of their second gestation.

Another group of sexually mature heifers, free from Bang's disease, were bred at such intervals that they would be in different periods of gestation at the time the udderless cows were in their fifth to sixth month of pregnancy. Shortly after the cows had been bred for their second gestation, the heifers were added to the group and all were held in the same pasture until 30 days after termination of the last pregnancy.

EXPERIMENTAL ANIMALS

The female cattle used in this experiment were assembled when 4 to 8 months of age, and represented different dairy breeds.

Group U–I (udderless infected) consisted of heifers nos. 24, 30, 31, 32, 34, and 47.

Group U–C (udderless controls) was composed of heifers nos. 33 and 35.

The udders and supramammary lymph glands of these eight heifers were removed surgically when the animals were approximately 6 months of age. These cattle showed a negative agglutination reaction for Bang's disease in monthly tests until they were 22 months old. At that time the heifers of group U–1 were experimentally infected with *Brucella abortus*. The heifers of the U–C group were not exposed to the infection.

Group N–C (normal controls) consisted of six heifers, nos. 2, 3, 4, 5, 6, and 7. The udders of these heifers were intact. The animals were aproximately 8 months old when assembled for this experiment and were about 1 year younger than those of the other groups. These heifers gave negative reactions in agglutination tests for Bang's disease.

Bull A, a grade Holstein of limited service as a sire prior to this experiment, was used in 1932 for breeding the udderless heifers. The animal gave negative reactions to the agglutination test for Bang's disease both before and at the conclusion of his use in this experiment. Bull B, a pure-bred Jersey, was obtained as a yearling from a negative herd. The animal, not used as a sire until about 30 months of age, was employed for all breeding services in this experiment during 1933. This bull gave negative agglutination reactions throughout the experiment.

ASSOCIATION OF GROUPS

The female cattle were pastured during the summer, and stabled with suitable lots for exercise during the winter. They were kept under conditions intended to protect them from exposure to Bang's disease until the time of experimental infection or use as controls for the presence of *Brucella* infection.

The animals of groups U–I and U–C were pastured and stabled together from June 1931 to October 1932. The heifers of these groups were bred during April, May, and June 1932. On October 23, 1932, the animals of group U–I were experimentally infected with cultures of *Brucella abortus*. Immediately prior to this infection the group U–C heifers were moved to the quarters of the group N–C animals.

Following infection each cow of group U–I aborted. These cows were bred again during April and May 1933.

The group U–C cows calved normally and were in direct association with the heifers of group N–C from October 1932 to June 1933, at which time all groups of the experiment were combined. The cows of group U–I had been bred before the groups were united. The cows of the other two groups, with the exception of no. 6 in group N–C (which had been bred in April), were bred at some time during the interval from June to September 1933. After the combination of the three groups the cows were maintained as a unit until the termination of the experiment in August 1934.

EXPERIMENTAL INFECTION

Three strains of *Brucella abortus*, each isolated in 1927 from aborted bovine fetuses, were used for infecting the cows in group U–I. Since their isolation the organisms had been maintained by monthly transfer on nutrient agar media and grew readily under ordinary atmospheric conditions at 37° C. The cultures were identified as bovine strains of *Br. abortus* by the use of dye media as suggested by Huddleson (*17*).

The virulence of the cultures was tested by guinea pig inoculations. The inoculum was a saline suspension of a 48-hour growth of the particular organism on liver agar media and was standardized to the opacity of no. 3 McFarland nephelometer tube. Three guinea pigs

were inoculated with each culture; one received a drop of the inoculum in the right conjunctival sac, another 0.5 cc intraperitoneally, and the other 1.0 cc subcutaneously. The guinea pigs were killed 6 to 8 weeks after inoculation, and on autopsy all but one showed splenic lesions from which *Brucella abortus* was isolated. The agglutinin titers of blood serum from these guinea pigs at the time of death ranged from 1:640 to 1:3,200.

Liver agar slopes were inoculated with the three strains of *Brucella abortus* and incubated 72 hours at 37° C. The growth was removed with sterile saline solution and the suspension adjusted to the opacity of a no. 8 McFarland nephelometer tube. Cows 24, 32, and 34 each received a vaginal inoculation October 23, 1932, of 30 cc of this suspension. The injection was made through a funnel attached to small rubber tubing, the outlet being carried well forward into the vaginal cavity. The inoculum was allowed to gravitate slowly into the vagina, but the entire amount injected was not retained. Cows 30, 31, and 47 each received a conjunctival instillation of two or three drops of the bacterial suspension. Following these inoculations the six cows were allowed to associate in an outdoors enclosure and an adjacent stable. At the time of abortion the cows were put in separate stalls, where they remained until all visible vaginal discharge had ceased. When an abortion occurred in the outside enclosure, the fetus and detached membranes were removed. No other precautions were taken to prevent reinfection of the cows from these sources.

The experimentally infected cows each aborted, within periods of 51 to 95 days after inoculation (table 1). Culture medium was inoculated and guinea pigs were injected with material from the abomasum of each aborted fetus. *Brucella abortus* was recovered from the fetuses of cows 24, 30, 31, and 47.

TABLE 1.—*Breeding and calving records of cows in groups U–I (udderless infected), U–C (udderless control), and N–C (normal control)*

Group and cow no.	Bred	Aborted	Calved	Bred	Calved
U-I:					
24	June 17, 1932	Dec. 13, 1932		May 1 and 22, 1933	Feb. 17, 1934
30	June 8, 1932	Jan. 7, 1932		Apr. 27, 1933	Feb. 10, 1934
31	May 10, 1932	Dec. 30, 1932		May 16, 1933	Feb. 16, 1934
32	June 3, 1932	Jan. 21, 1933		May 6 and Sept. 13, 1933.	June 23, 1934
34	May 20, 1932	Jan. 31, 1933		May 3, 1933	Feb. 7, 1934
47	June 4, 1932	Dec. 10, 1932		May 12, 1933	Feb. 25, 1934
U-C:					
33	Apr. 21, 1932		Feb. 1, 1933	Oct. 12, 1933	July 21, 1934
35	May 4, 1932		Feb. 15, 1933	Aug. 8, 1933	May 18, 1934
N-C:					
2				Aug. 4, 1933	May 15, 1934
3				Sept. 20, 1933	July 8, 1934
4				Aug. 26 and Sept. 17, 1933.	(1)
5				July 1, 1933	Apr. 9, 1934
6				Apr. 30, 1933	Feb. 4, 1934
7				June 9, 1933	Mar. 24, 1934

1 Did not conceive.

The group U–C, noninfected cows, produced apparently healthy calves at the termination of normal gestation periods.

Before the U–I group of cattle was bred for a second pregnancy, culture medium was inoculated with vaginal material collected from each cow by means of swabs. *Brucella abortus* organisms were not

recovered from any of the inoculations. These cows were bred prior to June 1, 1933, on which date they were turned to pasture in association with the U–C and N–C groups. Cow no. 32 failed to conceive from the service prior to being turned to pasture and was rebred in September. The cows of groups U–C and N–C were bred after the three groups had been combined.

After combination of all groups the cattle were maintained as a single lot until the termination of this experiment in October 1934.

Each of the cows, except no. 4 of group N–C, which failed to conceive, produced an apparently healthy calf at the end of a normal gestation period. Five of the cows in group U–I calved in February 1934, at which time the cows of the other groups were in different periods of gestation, one being in the fourth, two in the sixth, and one each in the seventh, eighth, and ninth month.

AGGLUTINATION TESTS

Results of tests for *Brucella abortus* agglutinins in the blood serum of the various animals, collected at approximately monthly intervals throughout the experiment, are given in table 2. The serums were diluted with antigen so that each tube in the series represented a dilution double that of the preceding. The original dilution was 1:25 and the series of dilutions continued until the agglutinating limit of the serum was reached.

TABLE 2.—*Agglutination records of the cows in groups U–I (udderless infected), U–C (udderless control), and N–C (normal control)* [1]

| Group and cow no. | 1932 | | | | | | | | | | |
	Feb. 4	Mar. 12	Apr. 16	May 15	June 23	July 25	Aug. 19	Sept. 19	Oct. 23 [2]	Nov. 20	Dec. 18
U–I:											
24	0	0	0	0	0	0	0	0	0	1,600	800
30	0	0	0	0	0	0	0	0	0	800	800
31	0	0	0	0	0	25–	25–	25	0	1,600	3,200
32	0	0	0	0	0	0	25–	25–	0	800	400
34	0	0	0	0	0	0	0	0	0	400	400
47	0	0	0	0	0	25	25	0	0	400	1,600
N–C:											
7	0	0	0	0	0	0	0	0	0	0	25–

| Group and cow no. | 1933 | | | | | | | | | | | |
	Jan. 19	Feb. 17	Mar. 18	Apr. 18	May 18	June 18	July 18	Aug. 21	Sept. 20	Oct. 20	Nov. 19	Dec. 21
U–I:												
24	3,200	6,400	3,200–	400–	200–	100–	200–	200–	100–	100–	100	100
30	12,800	6,400	6,400	800	800	200–	800–	800	400–	800–	800–	400–
31	12,800	6,400	400–	800	1,600–	400–	800–	800	400–	800–	200–	400–
32	3,200	6,400	1,600	800–	400–	100–	400–	400	400–	100–	200–	200–
34	12,800	12,800	12,800	3,200–	6,400	400–	800–	800	400	400	800–	800–
47	3,200	6,400	400–	400–	400	200–	400	400	200–	200	400–	200
U–C:												
33	0	0	25–	0	25–	25–	0	0	0	0	0	0
N–C:												
2	25–	25	25–	25–	0	0	0	0	0	0	0	0
3	25–	0	25–	0	0	0	25–	0	0	0	0	0
4	25–	25–	0	0	0	0	0	0	0	0	0	0
5	25–	0	0	0	0	0	0	0	0	0	0	0

[1] Figures alone represent maximum serum dilutions in which there was complete agglutination. The minus sign (–) following a figure indicates incomplete agglutination in that dilution.
[2] Each cow in group U–I was given a vaginal inoculation of *Br. abortus*.

TABLE 2.—*Agglutination records of the cows in groups U–I (udderless infected) U–C (udderless control), and N–C (normal control)*—Continued

Group and cow no.	1934							
	Jan. 21	Feb. 18	Mar. 18	Apr. 20	May 18	June 19	July 21	Aug. 18
U–I:								
24	200—	100—	100	100	100—	100—	200—	
30	1,600—	400—	400	400—	800	200—	200—	
31	800—	400	400—	400—	400—	200	400	
32	200—	100	200—	200—	200—	200—	200—	
34	800	800	800—	400—	400—	200	200	
47	400—	200—	200—	200—	200—	100	200	
U–C:								
33	0	0	0	0	25—	25—	25—	
N–C:								
2	0	0	25—	25—	0	25—	25—	0
3	0	0	0	25—	25—	25—	25—	25—
5	0	25—	0	25—	25—	25—	25—	25—
6	0	0	0	0	25—	0	25—	25—
7	0	0	0	0	0	25—	25—	25—

The antigen used in these tests represented three strains of *Brucella abortus*. Forty-eight-hour growth of these organisms on liver agar slants was removed with phenolized saline solution and the suspension standardized to the opacity of the no. 1 tube of a McFarland nephelometer.

Animals of group U–I did not show agglutinins in their serums, except in a few instances in the 1:25 dilution, until after the animal had been experimentally infected with *Brucella* organisms. Fourteen days after exposure the agglutinin titer of the infected cows ranged from 1:400 to 1:1,600 (not shown in table 2). The peak of these reactions was reached about 60 days after infection, at which time some titers were 1:12,800. During the succeeding 18 months there was a gradual, though somewhat irregular, recession in the limit of the titers. No animal at any time showed a titer lower than incomplete in 1:100; and at the conclusion of the experiment, 21 months after experimental infection, the titers ranged from incomplete in 1:200 to complete in 1:400.

The cows of groups U–C and N–C usually gave completely negative tests. Although agglutination was observed at different times in the 1:25 dilution to some of the blood samples, these reactions were never complete. Failure of these control animals to develop high agglutinin titers was not considered as due to inability of response to antigen stimulation, since four of the cows that later received vaginal inoculations of *Brucella abortus* cultures developed high titers.

Agglutination tests of milk samples obtained from separate quarters of the udders and collected from each cow of the N–C group about 1 week following her calving, were negative. After all the cows of this group had calved, milk was again collected from each for agglutination tests and guinea pig injections. Each sample represented a mixture of milk from all four quarters of the udder. Negative agglutination reactions were obtained with these samples.

Two guinea pigs were inoculated, one intraperitoneally and the other subcutaneously, with gravity cream from milk of each cow. Seven weeks after these injections the guinea pigs were killed for autopsy. Evidence of *Brucella abortus* infection was not obtained in

any of these animals either by post-mortem examination, inoculation of culture medium with spleen tissue, or agglutination tests with blood collected from the guinea pigs at the time of slaughter.

DISCUSSION

A review of the literature shows that some but not all reactor cows having udder infection eliminate *Brucella abortus* in placental membranes at the termination of gestation. Whether reinvasion of the uterus results from the udder infection of the individual cow or comes from other sources, seems not to have been established.

Obviously it is impossible to prove that udder infection would have resulted from the inoculations of *Brucella abortus* given the udderless cows used in this experiment. However, the results obtained by the writer in another test, wherein two of four heifers, aborting after vaginal inoculation with subcultures of the same strains of *Brucella abortus* as were used in the present experiment, showed udder infection, appears to warrant the assumption that at least some of the udderless heifers would have shown udder infection if their udders had not been removed.

The animals used in this test were protected against sources of *Brucella abortus* infection other than the experimental inoculation, but no effort was made to isolate the individual animals as protection against a possible intragroup spread of infection. That the protection was adequate for the conditions of this experiment is apparent from the fact that evidence of *Brucella abortus* infection was not obtained from any of the animals during the period of their association as a single herd.

The significance of agglutinins in the 1:25 dilution of blood serum of the control animals may be open to question, since the minimum titer indicative of infection is a somewhat controversial point. The United States Live Stock Sanitary Association (*26*) recommended for the control of Bang's disease in farm animals, that cattle having agglutinin titers less than complete in the 1:25 dilution be classified as negative, and that those with complete agglutination in the 1:100 or higher dilutions, be considered as positive. Animals giving reactions between these limits are classed as suspicious. The success obtained with measures for abortion control in numerous herds representing thousands of animals, in which agglutination in the 1:25 dilution is not regarded as indicating infection of the animal, seems to justify the opinion that agglutinins in that dilution alone rarely if every indicate an infection of the animal. The failure of the control heifers in any instance to show an increase in agglutinin titers for at least 90 days after the demonstration of agglutinins in the 1:25 dilution tends to support the opinion that active infection was not present in these animals.

Whether the recession but continued maintenance of agglutinin titers within the generally accepted reactor range shown by the experimentally infected cows following abortion, represented a maintained agglutinin production by animals that no loger harbored the infection or whether it was the result of residual infection in some part of the body other than the udder and supramammary lymph glands, remains a question. If the latter is true the cows evidently were not

disseminators of infection during their second gestation period, as judged by nonoccurrence of abortion, nonoccurrence of abortion in the pregnant control heifers directly associated with the udderless reactor cows, failure of agglutinins to develop to a titer generally accepted as indicating infection, negative results of cultural tests with milk and vaginal material collected from these heifers and vaginal material obtained from the udderless cows.

SUMMARY

The object of the experiment herein reported was to determine whether the reactor cow that has aborted but has no udder infection may disseminate *Brucella abortus* during her subsequent gestation.

Cows whose udders and supramammary lymph glands had been removed surgically during calfhood were used in the experiment to eliminate any possibility of the presence of nondetectable *Br. abortus* udder infection in the test animals.

Removal of the udder did not affect gestation, as was demonstrated by the fact that noninfected udderless cows delivered normal calves.

Abortions were induced in udderless heifers by vaginal inoculation with *Br. abortus* (bovine) organisms.

The results give conclusive evidence that the presence of *Br. abortus* agglutinins in the blood of cows is not dependent upon specific udder activity, since each of the infected udderless cows developed a high agglutinin titer. While the height of these titers gradually subsided during the latter part of the experiment, it was always maintained at a point, 1:100 or above, which justified the classification of the animals as positive reactors.

These reactor cows did not eliminate *Brucella* organisms during their second gestation as judged (1) by nonrecovery of organisms from vaginal material of the udderless cows collected within a few hours after their second calving; and (2) by failure of susceptible heifers associated with the udderless cows to abort, to develop specific agglutinins in their blood or milk, or to show *Brucella* organisms in milk or vaginal material collected soon after their calving.

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ROUGH SALMONELLA PULLORUM VARIANTS FROM CHICKS [1]

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INTRODUCTION

Morphological variants among bacteria have been observed by many investigators. The earliest views on variation were crystallized by Nägeli (*4*).[2] Hadley's (*1*) review on "dissociation" leaves no doubt that morphological variability occurs in most bacterial species.

RELATED INVESTIGATIONS

Studies on *Salmonella pullorum* variants were made by Mallmann (*3*), who classified stock cultures into three types; viz, stable smooth, stable rough, and variable rough-smooth or intermediate. A description of these was not given.

Plastridge and Rettger (*6*) have reported on a pleomorphic *Salmonella pullorum*-like organism which was isolated from chicks, adult birds, and eggs laid by the adults that survived a disease outbreak. They found this organism to have fermentative and antigenic characteristics similar to those of *Salmonella pullorum*. They said however, "* * * it differs materially from the well known pullorum disease organism in morphology, cultural requirements, and virulence for adult birds." They (*7*, p. [*146*]) also stated that the organism must be considered as a "* * * variant of S. *pullorum* or unusual virulence. This conclusion is further confirmed by the fact that some of the strains isolated from infected birds gradually lost their pleomorphism * * *." The virulence of this organism, however, remained at a comparatively high level even after 6 months' cultivation under artificial conditions. After studying the effect of bacteriophage on 10 stock-culture strains, they reported observations on 3 principal colony types grown on beef-infusion and liver-infusion agar, viz, S_1, ordinary smooth colonies; S_2, colonies that were smooth in appearance but much larger than S_1 colonies; and R colonies, which possessed distinctly roughened surfaces.

The present paper presents data on two rough *Salmonella pullorum* variant strains isolated from chicks.

EXPERIMENTAL SUBJECTS AND PROCEDURE

Three 7-day-old chicks taken from a brood of 240 were sent to the laboratory for autopsy. The house in which the chicks were brooded had not previously been used by poultry for 5 years. This brood of chicks was purchased from a hatchery which obtained eggs from 11 supply flocks. These supply flocks were all officially State blood-tested (stained *Salmonella pullorum* antigen) for pullorum disease

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carriers before the eggs were collected for hatching purposes. Adult birds in four of these flocks gave a negative reaction in the first test, two flocks gave a negative reaction in the second test, and five flocks were found to contain reactors in the second and final test.

On post mortem the following findings were noted in all three chicks: Yolks—partially absorbed; kidneys—swollen, congested, and containing an abnormal amount of urates; lungs—congested; ureters—distended with urates; proventriculi—thickened mucosa and swollen glands; gizzards—linings eroded and slightly pitted; livers—slightly enlarged, congested, and showing a peripheral gray to yellow pin-point mottling.

Liver tissue from each of the three chicks was streaked to routine nutrient agar (pH 6.8). This medium is known to be satisfactory for the isolation and support of normal smooth stains of *Salmonella pullorum*.

EXPERIMENTAL RESULTS

After 24 hours incubation at $37\frac{1}{2}°$ C. the agar slants streaked with liver tissue from chicks nos. 1 and 2 showed a few minute colonies, either well-isolated or protruding from a thin, delicate, dull-gray coating of growth which covered a small portion of the agar surface. After failing to show growth at 24, 48, and 72 hours of incubation, the agar slant inoculated with liver tissue from chick no. 3 was discarded.

To facilitate a morphological study, strains 1 and 2, from chicks 1 and 2, respectively, were streaked to a series of Petri dishes containing nutrient agar, and incubated for 24 hours at $37\frac{1}{2}°$ C. The plates were relatively free from condensation fluid.

After the inoculated plates had been incubated for 24 hours, strains 1 and 2 appeared indentical. A very thin, delicate, lusterless gray growth was present in areas where the bulk of inoculum had been placed. This growth was seen more clearly when the plates were tilted toward the light. A few small, distinct colonies were also present, some well-isolated and others protruding from the delicate basic growth. These colonies were 0.4 mm or less in diameter and appeared dull gray in reflected light.

A microscopic examination of the cultures ($\times 100$ transmitted light) showed the thin, delicate, lusterless gray growth to consist principally of minute, extremely irregularly shaped colonies of various sizes from which projected strands of various lengths and shapes. Frequently single, well-isolated strands constituted a colony. The majority of these colonies were microscopic in size, but a few of the larger ones were barely visible to the naked eye. For convenience in discussing these colonies, the extremely irregular ones are designated the XXR type, and the larger, less irregular, veined colonies, the RRR type. Plate 1, *A* and *B*, shows several typical XXR colonies, and plate 1, *A* and *C*, shows the RRR type. The RRR colonies varied from 0.3 to 0.4 mm in diameter. The borders were more or less wavy and the colony surface distinctly irregular, veined, and granular (transmitted light).

Smears from 24-hour-old XXR colony types, stained with dilute carbolfuchsin, showed extreme chain forms or filaments (pl. 1, *D*). Single rod-shaped cells were present, but chain forms predominated in the microscopic field. Many of these chains extended across several microscopic fields ($\times$ 950). Bipolar staining was sometimes

PLATE I

Typical colonies and cells as viewed by transmitted light: *A*, XXR and RRR colonies, × 100; *B*, XXR colony, × 100; *C*, RRR colony, × 100; *D*, cells from XXR colony, × 1,800; *E*, cells from RRR colony, × 1,800.

observed and when present was confined principally to the single cells. However, the uneven staining sometimes seen in the bacterial chains suggests the possibility of bipolar staining or dead bacterial cells within the chains. Single cells varied in size from 0.92μ to 2.6μ · in length, and from 0.33μ to 0.54μ in width. The width of the chains varied from 0.33μ to 0.55μ.

Smears from RRR colony types after 24 hours' incubation also showed chain formation (pl. 1, *E*), but they did not show the extremes noted in smears from XXR colonies. Single and double rods in these colonies were more numerous than in smears from XXR colonies. Single rods measured 0.85μ to 2.4μ in length, and 0.3μ to 0.5μ in width. The width of the chains varied from 0.3μ to 0.53μ. Bipolar staining was noted in some of the single and double cells, and an uneven staining in some of the chains.

Organisms from XXR and RRR colony types were gram-negative, nonmotile, and did not produce indole. When tests for H_2S production were made by the method of Jordon and Victorson (*2*) a slight blackening of the culture medium along the line of inoculation was noted after 72 hours' incubation.

Cells from both colony types were inoculated to Dunham's fermentation tubes, such tubes containing 0.75 percent of the test carbohydrate and 1 percent of Andrade's indicator. The inoculated tubes were incubated for 14 days at $37\frac{1}{2}°$ C. and produced both acid and gas in tubes containing mannitol, dextrose, arabinose, xylose, rhamnose, levulose, mannose, galactose, and sorbite. Neither acid nor gas was formed in media containing lactose, maltose, sucrose, raffinose, dextrin, inulin, dulcitol, erythritol, salicin, and inositol.

To test the variants under study for autoflocculation, a phenomenon observed by Nicolli (*5*), saline emulsions of a mixture of cells from XXR and RRR colony types were prepared in concentrated and dilute suspensions. These suspensions contained 0.9, 0.5, and 0.1 percent NaCl, respectively, and were preserved with 0.3 percent phenol. After incubation for 40 hours at $37\frac{1}{2}°$ C. autoflocculation was not observed, although a slight cell sediment was noted in all tubes, including control tubes, containing the same cell mixture in phenolized distilled water. Upon agitation the sediment arose in a cloudy spiral and became evenly distributed in the test fluid.

To test the agglutinability of the variants an antigen was prepared by suspending a mixture of cells from XXR and RRR colony types in a saline solution containing 0.9 percent NaCl and 0.3 percent phenol. The antigen was adjusted to a turbidity of 1.0 of McFarland's nephelometer scale. After 40 hours' incubation at $37\frac{1}{2}°$ C. the antigen was completely agglutinated by a known positive *Salmonella pullorum* serum to a titer of 1 to 400. Partial agglutination occurred in the 1 to 800 and 1 to 1,600 dilutions, but agglutination was not observed in the 1 to 3,200 dilution.

When a similarly prepared antigen was run with negative fowl serum, a distinct clearing, equivalent to a 3+ reading in true agglutination, was noted in dilutions up to and including 1 to 200, 2+ in 1 to 400, and 1+ in 1 to 800 and 1 to 1,600. The partial clearing in the tubes, however, could not be attributed to true agglutination, for on agitation the cell sediment became resuspended without evidence of clumping.

SUMMARY

Studies are presented on two variant strains of *Salmonella pullorum* isolated from baby chicks. These strains varied morphologically and serologically from the normal smooth *S. pullorum* strains usually found in chicks that succumb to the bacteriemic form of *S. pullorum* infection. Both strains exhibited a similar rough colony morphology characterized by two distinct colony types (XXR and RRR types). Antigens prepared from both strains were agglutinated by *S. pullorum* serum and were sensitive to and only partially stable in a saline-negative serum mixture.

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LETHAL HIGH TEMPERATURES FOR CONIFERS, AND THE COOLING EFFECT OF TRANSPIRATION [1]

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INTRODUCTION

Studies of lethal temperatures for conifers have been confined mostly to experiments on young seedlings, and have been carried out chiefly with the object of determining the temperatures at the soil surface which cause stem lesions or "white spot" injury (9).[2] The results show considerable variation, depending upon the methods used and the duration of exposure to heat.

In general, heat injuries most frequently occur to plants exposed to intense insolation. Because leaves are most exposed to direct sunlight they are more commonly injured than other parts once the plant is past the juvenile stage. The purpose of the present study was to determine accurately the lethal high temperatures for the species most widely used in forest planting in the Lake States, not only with the view of obtaining a clue to success or failure in plantations, but also to find the highest safe temperature at which tests of drought resistance might be conducted. It was considered of especial interest to determine whether the cooling effect of transpiration enables plants to withstand a higher temperature at low humidities than in air of high moisture content.

RELATED INVESTIGATIONS

That excessively high temperatures often limit the success of both natural and artificial regeneration of conifer forests has been shown by observations made at the Lake States Forest Experiment Station, and is well attested by the literature. Temperatures of surface soil up to 55° C. have been reported in nurseries by several investigators. The writer has measured a temperature of 53° in the Forest Service nursery at Cass Lake, Minn., and even higher temperatures have been reported from field stations. Toumey and Neethling (20) found at Keene, N. H., that during early July maximum temperatures of surface soil in open fields and small openings in the forest were rarely lower than 40°, and in more than 50 percent of the cases observed they were in excess of 50°. On warm days it was not unusual for the soil temperature to remain above 50° for 6 consecutive hours. Similar high temperatures were observed by Li (12). On the Huron National Forest in Michigan, surface temperatures in sand of 54° to 72° have been observed. Even at a depth of 1 inch, the soil temperature attained as high a value as 55°. Bates and Roeser (4) and Roeser (17) report soil temperatures of 67° to 70° on south slopes of the Rocky Mountains in Colorado.

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ON LETHAL HIGH TEMPERATURES

According to Miller (*15*), the actual killing temperature, or thermal death point, for most plant cells has been found to vary between 45° and 55° C.

There is considerable variation among species of plants in the degree of heat that they are able to withstand (*13*). The amount of injury at a given temperature increases with increasing length of exposure, and the higher the temperature, the shorter the exposure required to produce death or a given degree of injury. The thermal death point may be altered by an increased concentration of salts in the cell sap, which raises the coagulation point of colloids.

Clum (*7*), by the use of minute thermopiles which could be inserted in the leaf tissue, found that injury to leaves of lilac (*Syringa vulgaris* L.) and fuchsia (*Fuchsia* sp.) occurred at temperatures of 50° C., while privet (*Ligustrum vulgare* L.) was somewhat more resistant. Arthur and Stewart (*1*) noted severe injury to tobacco (*Nicotiana tabacum* L.) leaves at 53°. Berkley and Berkley (*5*), in an exhaustive study of killing temperatures for cotton plants (*Gossypium* sp.), found the lethal temperature to range from 40° to 84°, depending on the age of the plants and the duration and conditions of exposure.

Heat injuries to North American conifers are not uncommon in nursery beds, where white spot or stem lesions (*9*) may frequently be found at or near the ground surface. Hartley (*9*) found that stem lesions of coniferous seedlings occurred at 52° C. Bates (*3*) studied the resistance of seedlings of lodgepole pine (*Pinus contorta* Dougl.), ponderosa pine (*Pinus ponderosa* Dougl.), Engelmann spruce (*Picea engelmannii* (Parry) Engelm.), and Douglas fir (*Pseudotsuga taxifolia* (Lamb.) Brit.) to excessive temperatures of surface soil when exposed to intense solar and artificial radiation. Accurate control of temperature was not maintained. From these tests he concluded that injury is greatest to the youngest seedlings; seedlings which have survived a definite exposure to excessive heat are not likely to be injured by subsequent exposures of the same severity.

Further studies of the same species by Bates and Roeser (*4*) and by Roeser (*17*) under similar conditions of exposure, as well as of other conifers by Baker (*2*) and by Toumey and Neethling (*20*), confirm the conclusion that resistance to heat increases with increasing age of the plants during the first year of life. Data presented by Bates and Roeser (*4*) indicate quite clearly that the degree of injury increases with increasing time of exposure. Exposures of 30 minutes or longer in air temperature as low as 49° C. caused some injury, while 13 minutes' exposure at 58° caused no injury. Some plants successfully withstood temperatures even up to 83° for a period of 7 minutes.

Toumey and Neethling (*20*) exposed echard pans containing seedlings of Norway pine and white pine 2 to 12 days old to solar radiation through a window, supplemented by radiation from an electric heater. Air temperature within a single test varied from about 36° to 39'° C. Heat lesions were caused by soil temperatures of 49.4° to 50.6° when exposure time was as long as 2 hours. Under the conditions prevailing in their test it was impossible to regulate soil temperature accurately, and the results consequently showed deviations.

Baker (*2*) carried out a rather comprehensive series of tests of lethal high temperatures on a number of western conifers. Young seedlings, 1 to 3 months old, with thermocouples inserted in the stem at the

ground line, were exposed in shallow pans to radiation from a radiant electric heater. As soon as definite signs of injury were observed a test was terminated. The actual temperatures involved were determined by thermocouples placed in the air and surface soil. Considerable variation in temperature existed in all tests. The seedlings used were quickly killed when the internal temperature reached 54° C., but temperatures only a few degrees lower were endured for a considerable time. In this respect there was relatively little difference among the species used. A more significant difference among species was the differential between soil temperature and stem temperature, which was as great as 8° to 11° for the coarser stemmed seedlings. This difference between soil temperatures and internal temperatures may explain in large part the inconsistencies reported by other investigators.

Roeser (*17*) tested the heat resistance of coniferous seedlings in another way. Seedlings approximately 58, 71, and 110 days old (calculated from date of sowing) were strung through a board with their roots in water. On this board heated fine sand was sprinkled to a depth of one-fourth inch. A thermometer on the board was used to determine the actual temperature to which the seedlings were exposed. There was considerable difference among the different species used. Fifty-eight-day-old seedlings of ponderosa pine withstood temperatures up to 70° C., Engelmann spruce to 63°, and lodgepole pine only to 59°, while 39-day-old Douglas fir withstood temperatures up to 66°. Variations in killing temperature among different individuals of the same species and age class were as much as 8° to 27°. The most resistant of all, 110-day-old ponderosa pine, withstood temperatures up to 83°.

The only paper reviewed which deals with the effect of high temperatures on conifers beyond the cotyledon stage is that of Korstian and Fetherolf (*10*), who found that 3–1[3] Engelmann spruce transplants were severely injured and in many cases killed by excessive heat, which killed the cambium at the ground line. These injuries were observed in forest nurseries where the actual temperatures involved could not be determined accurately. The highest temperature measured was 54° C.

ON THE INFLUENCE OF TRANSPIRATION ON THERMAL DEATH POINT

There has always been considerable controversy as to whether transpiration plays an important role in reducing leaf temperature. If it does, plants might be expected to withstand a higher external temperature under conditions which favor rapid transpiration. The comprehensive studies of Clum (*6, 7*) on the temperature of leaves under various conditions of atmospheric temperature, humidity, and light led him to conclude that transpiration plays only a minor role in reducing the temperature of leaves. For the plants studied he found normal leaves to be only 2° to 4° C. cooler than leaves coated with petrolatum or leaves of plants growing in dry soil. He was unable to establish any relationship between leaf temperature and transpiration rate.

Copeland (*8*) made a determination on the cooling effect of transpiration with leaves of *Ceanothus velutinus* Dougl. and other chaparral species, by measuring the leaf temperatures under conditions

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[3] Transplants 3 years in the seedbed and 1 year in the transplant bed.

favoring transpiration and under conditions of minimum transpiration. The technique used was to wrap the leaf around a mercurial thermometer and expose it at normal incidence to the sun's rays. When the upper, stomata-free surface, or the undersurface coated with petrolatum was exposed, the temperature was 8° to 11° C. higher than when the uncoated undersurface was exposed. This difference he attributes mainly to transpiration. Leaves which transpired more slowly showed less difference in temperature under similar conditions of exposure.

Miller and Saunders (*16*) found the temperature of wilted leaves in the sun to be from 1.55° C. (for those of sorghum plants) to 4.65° (for those of cowpeas) higher than that of turgid leaves under similar conditions of exposure. The leaves which showed the greatest difference in temperature between the wilted and turgid conditions also had the greatest difference in transpiration.

By thermodynamic studies on leaves in air, Watson (*21, 22*) was able to formulate a law of thermal emissivity for leaves. Thermal emissivity, or the giving off of heat by the leaf, includes the heat dissipated by radiation and by conduction to the surrounding air, but not the heat used in the transpiration process. From his own studies and those of Meyer (*14*) on transpiration, Watson concluded that thermal emission is of importance in accounting for the loss of heat from leaves. Since the rate of thermal emission increases rapidly with the temperature differential between the leaf and the surrounding air, he is of the opinion that this factor is the deciding one in preventing the thermal death point being reached under conditions of intense insolation. Watson in his study assumed that leaves obey the theoretical laws of thermodynamics. This is probably so within narrow limits, but under extreme conditions the relative importance of transpiration and thermal emission may be reversed.

Arthur and Stewart (*1*), working with tobacco plants, showed that at high temperatures a coating of petrolatum, such as was used by Watson and others to eliminate transpiration, does not really diminish transpiration to any appreciable extent in tobacco leaves. These workers studied the transpiration rate of tobacco plants when exposed to intense radiation from a 1,000-watt lamp. As the distance between the plants and the lamp decreased, the temperatures of the leaves rose slowly to a maximum of 42° C. With further increase in radiation intensity there was an increase in transpiration but no increase in leaf temperature. However, when a tobacco leaf was enclosed in a tight-fitting cellophane envelope and exposed under similar conditions, the leaf temperature rose within 4 minutes to 53°, and severe injury resulted. From these studies the authors concluded that transpiration of leaves is the factor which makes it possible for them to exist under conditions of high radiation intensity and high temperatures. However, it should be pointed out that the cellophane envelope not only surrounds the leaf with a saturated atmosphere, but also reduces to a considerable extent possibilities of thermal emission to the outside air.

Some observations on the effect of atmospheric humidity on the killing temperatures of conifers were made by Bates and Roeser (*4*), who heated plants in an incubator with approximately saturated air. The temperatures which caused severe injury and death to the plants varied from 61° to 66° C. in the saturated atmosphere, and from 66°

to 83° in normal atmosphere. The lengths of these exposures varied from 1 to 10 minutes in saturated air and from 5 to 14 minutes in normal atmosphere.

Roeser (*17*) found that ponderosa pine and lodgepole pine transpired approximately 13 times more water at 51° C. than at 16°, while for Douglas fir at the same temperatures the increase was 18 times, and for Engelmann spruce, 28 times. There was some difference among species in the promptness with which the increase in transpiration followed an increase in temperature. Generally speaking, the ones which most promptly responded to elevation of temperature with increase in transpiration were more resistant to excessive heat. Roeser's work does not, however, present a clear-cut case for the protective value of transpiration.

The most significant work on the effect of humidity on lethal temperatures is that of Berkley and Berkley on the cotton plant (*5*). These workers took particular pains to control temperature to within ± 0.5° C., a much narrower range than was maintained in any of the experiments on conifers described above. They found that the time required to kill all plants varied with temperature. For an exposure of 1 minute the cotton plant was killed at 65° in moist air and 84° in dry air. Throughout the temperature range used there was a difference of at least 5° between the temperature required to produce death in moist air and that required to produce death in dry air. Since excessive heat was applied in the form of air temperature and not in the form of radiation, thermal emission could play no part, hence the excess in temperature required to produce death in dry air may be considered as due in large part to the cooling effect of transpiration. A secondary influence is the greater specific heat of moist air, which may have caused a more rapid heating of the plant tissues.

EXPERIMENTAL TECHNIQUE

The tests undertaken for this study were designed to determine the killing high temperatures for both roots and tops and the influence of variations in relative humidity upon these killing temperatures. No attempt was made to simulate field conditions. Exposures were made in a water bath, in moist air, and in dry air.

The four conifers most commonly employed in forest planting in the Lake States region were used in the tests: Norway pine (*Pinus resinosa* Solander), white pine (*Pinus strobus* L.), jack pine (*Pinus banksiana* Lamb.), and white spruce (*Picea glauca* (Moench) Voss). The plants used were from stock which had been growing in a greenhouse for at least 2 years. All stock less than 3 years of age was raised from seed in the greenhouse. During the summer months temperatures in the greenhouse were usually higher than outside air temperatures, so all plants used had probably survived exposure to air temperatures as high as 45° C.

THE WATER BATH

The plants were lifted from the greenhouse bench and sorted as nearly as possible into lots of equal health and vigor. Five plants of each species and age class were provided for each of the following three types of exposure: Roots only in water; tops only in water; plants completely submerged. The roots of plants exposed with tops only in water were packed in damp sphagnum moss to prevent drying.

Species and age class	Temperature [1]													
	52.8° C.	51.7° C.	51.3° C.	50.3 °C.	48.9° C.	48.6° C.	48.4° C.	48.2° C.	47.6° C.	47.1° C.	46.9° C.	45.9° C.	45.7° C.	44.3° C.
Norway pine:														
1-0														
1-1								x x	x	x	x		x x	
1-2		x	x	x	x			x x	x	x	x	x x	x x	
White pine:														
1-0														
1-1		x x	x	x	x x	x x		x x	x x	x x	x x	x x x x	x x	x
1-2	x													
Jack pine: 1-0	x	x x	x	x	x x	x x	x	x				x	x	
White spruce: 1-0														x

5-HOUR EXPOSURES

Species and age class	52.8° C.	51.7° C.	51.3° C.	50.3 °C.	48.9° C.	48.6° C.	48.4° C.	48.2° C.	47.6° C.	47.1° C.	46.9° C.	45.9° C.	45.7° C.	44.3° C.
Norway pine:														
1-0														
1-1							x x					x x		x x
1-2							x x x x					x x x x	x x	x x x x
White pine:														
1-0														
1-1							x					x	x	x
1-2														
Jack pine: 1-0														
White spruce: 1-0														

[1] "x" indicates that plants of the species and age class shown at the left were exposed at the temperature indicated.

In order to be assured that no injury would result from submergence in water as such, four tests of 2 hours duration and one of 5 hours duration were run with the plants exposed in similar manner in water at room temperature. All species and age classes were used in these tests, and in no case was any injury observed.

Following exposure, the plants were returned to the greenhouse and heeled in. After sufficient time had elapsed for injuries to be readily discernible, usually a week or more, each plant was dug up and carefully examined for injury to fibrous roots, main roots, stem, needles, and buds. Control plants were examined in the same manner.

The water bath used consisted of a double-walled, cork-insulated copper vessel, equipped with an electric stirrer and electric immersion heater, controlled by a sensitive mercury thermoregulator through a relay.[4]

The species of plants, age classes, and temperature conditions in the various tests are shown in table 1. Temperatures were read at 10- to 20-minute intervals for the 2-hour exposures and at 15- to 30-minute intervals for the 5-hour exposures. Variations in temperature were not more than 0.2° except for a slight drop when the plants were first introduced. Any significant deviations were corrected as soon as detected by adding hot or cold water.

EXPOSURE TO MOIST HEAT

For determining the killing temperatures for conifers in hot air of high relative humidity the apparatus developed by the writer for testing drought resistance was used (*19*). This so-called "drought machine" (fig. 1) consists of a cylindrical chamber 30 inches in diameter provided with electric lamps, a thermostatically controlled electric heating coil, and a revolving table for the plants. Air is forced through a "tower" of calcium chloride into the chamber. To obtain high relative humidity, the calcium chloride was replaced by a fine spray of hot water that brought the humidity up to 80 to 94 percent. Under these conditions droplets of water usually collected on the test plants. The radiation intensity from lamps alone at the average heights of the plants was measured with the Shirley radiometer (*18*). Illumination was measured with the Macbeth illuminometer. Wet- and dry-bulb thermometers were read at 15- to 30-minute intervals during the course of each test, and at the end of each test the soil temperature was determined from a thermometer thrust into the soil. For practically all tests temperatures were maintained within ±1.5° C., except for rather considerable drops, when the plants were first placed in the machine.

The conditions prevailing during the various tests, together with the species, number, and age of plants used are given in table 2. The temperature differential between tests was reduced near the critical value. Soil temperatures on 5-hour runs averaged about 2° C. below air temperatures. Relative humidities, except for the first two tests, averaged about 90 percent. With a few exceptions two radiation intensities prevailed—0.093 and 0.131 gram calories

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[4] The writer is indebted to Dr. R. B. Harvey, of the Department of Plant Pathology and Botany, University of Minnesota, for the use of the necessary laboratory equipment for conducting the submergence tests.

per square centimeter per minute. Three degrees of illumination were used—7.6, 17.1, and 28 footcandles.

The test plants had been grown in the greenhouse under the same conditions as those used in the submergence experiments, the 1–2
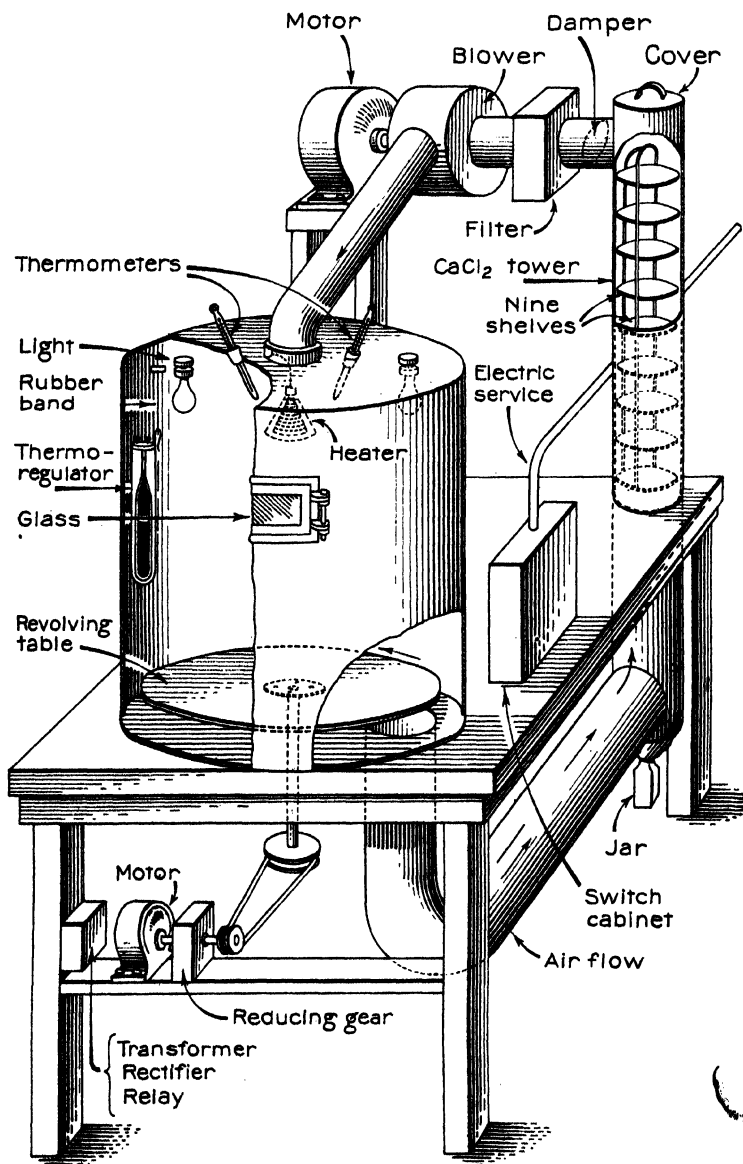
FIGURE 1.—The "drought machine." The trays were removed from the calcium chloride tower and replaced by a spray jet which provided a fine mist of warm water for producing high relative humidity.

Norway and white pines being from the same lot of plants. The plants tested were transplanted in a light sandy soil in 4-inch clay flowerpots or tin cans. All transplanting was done at least 2 weeks

TABLE I — Conditioning young conifers of different... 2-HOUR EXPOSURES

Num- ber		Num- ber	Age class	Num- ber		Num- ber		Maxi- mum °C.	Mini- mum °C.	Soil tem- pera- ture		Maxi- mum Percent	Mini- mum			Gram- calories per square cen- timeter per min.	Foot- candles
Norway spruce		Jack pine															
10	1–2	9	1–2					54.0	53.0	5		80.0	62.0			.131	28.0
10	1–2	14	1–2					50.2	49.0	4		95.0	75.0			.131	28.0
15	1–2	9	1–2					58.1	57.5	5		95.0	84.0			.131	28.0
9	1–2	15	1–2						51.5			95.0	89.0			.131	28.0
13	2–1	5	2–1					8	44.0			97.0	85.0			.131	17.1
												95.0	85.0				

2-HOUR EXPOSURES

				5		51.6	53.0	51.0				80.0	62.0				
9		0	1–1	5		58.8	60.5	55.0				95.5	75.0				
8		7	1–1	5		49.6	51.0	48.0			48.0	97.5	84.0				
11		3	1–1	5		49.9	50.2	49.5			50.0	95.0	89.0				
5		5	2–2	5		52.6	53.5	52.5			50.0	99.0	85.0				
		5	2–2			52.1	52.5	51.5				97.0	85.0				

2–1	5	2–2				51.4	52.0	50.0				95.0	74.0				
2–1	5	2–2				48.1	50.0	46.0				100.0	79.0				
2–2	5	2–2				47.9	49.0	47.0				92.0	84.0				
2–2	5	2–2				47.8	50.0	47.5				94.0	74.0				
2–2						48.9	50.0	0				95.0	70.0				

FOUR EXPOSURES for 20

prior to testing. The plants were removed from the greenhouse and thoroughly watered before being placed in the testing machine. At the end of the tests, the plants were returned to the greenhouse and watered again. A week or more later, the needles, buds, and stems were examined carefully to determine injury, if any. Roots of a few plants were examined but showed no injury.

To make certain that humidity alone or the testing procedure did not influence the results, one test of 16 hours' duration was run with all four species at 37° C. with the relative humidity averaging 97 percent. Under these conditions not a single plant was injured.

<div align="center">EXPOSURE TO DRY HEAT</div>

For tests in hot, dry air the drought machine (fig. 1) was operated in the regular manner. The plants were placed on the revolving table and illuminated with incandescent lamps. The air in the chamber was circulated through the calcium chloride tower two to three times per minute. Temperatures were maintained with the thermostatically controlled electric heater. Illumination was varied during these tests from darkness, i. e., radiation from electric heater only, mostly infrared (radiometer reading 0.12 gram-calories per square centimeter per minute, illuminometer reading 0.02 footcandles), to the light from two 300-watt clear incandescent lamps (radiometer reading 0.45 gram-calories per square centimeter per minute, illuminometer reading 306 footcandles).

The conditions prevailing during the tests and the types of plant used are given in table 3. Temperatures were held to within ±1° C. For most temperatures two to three tests were run. Results of tests that for any reason were not properly controlled were discarded in determining critical temperatures. Soil temperatures were from 5° to 20° lower than air temperatures in these tests.

Because of evaporation from the soil it was not practicable to reduce the humidity below about 15 percent. For 5-hour tests, although the maximum values were the same, the averages were much lower. The low humidities correspond with the average minimum values occurring in the field on hot, dry summer days in the Lake States region.

The plants were from the same lot as those used in preceding tests. The soil moisture in the pots was adequate but below the water-holding capacity, since with saturated soil it was impossible to keep the humidity down. Samples taken after each run showed that in almost every case there was at least 5 percent moisture in the soil, which is well above the wilting coefficient for the sandy soil used. Similar plants live from 1 to 3 weeks in the same soil at 2 percent moisture when exposed to continual drying in the drought machine.

After the run was completed the plants were returned to the greenhouse and left there a week or more before final examination.

Num-ber	A cla	Num-ber	Age clas.	Num-ber	Age class	Num-ber	A cla	Mi	Mini-mum	M m	Soil tem-pera-ture	Pcecnu	Maxi-mum	M m	Gram. calories per square cen-timeter per minute	Foot-andles
10	2-1	10	2-1		1			57.7	57.5	58.2	41.5	20.2	24.0	13.0	.126	49.0
10	2-1	10	2-1		0			54.8	55.0	54.0	42.0	23.4	27.0	17.0	.126	49.0
5	2-1	10	2-1		0			54.3	53.0	55.0		22.1	25.0	18.0	.126	49.0
9	2-1	11	2-1		1		1	54.0	53.0	54.5	35.0	23.2	31.0	18.5	.121	93.9
5	1-1				1			53.3	52.5	54.0	34.0	22.5	24.0	19.0	.121	49.0
4	2-1				1		1	53.2	52.5	53.5	38.5	17.3	21.0	12.0	.121	306.0
4	1-1				1		1	52.5	51.5	53.5	39.0	16.8	21.4	11.0	.451	(¹)
4	1-1				0		1	52.5	52.0	54.0		13.1	16.3	9.0		(¹)
10	2-1				0			52.3	48.0	53.0	35.0	21.2	26.0	14.0	.121	93.9
10	2-1				1		1	52.3	52.0	52.5	32.5	23.5	26.0	21.0	.121	93.9
5	2-1				1		1	51.5	50.0	52.5	33.5	22.5	28.0	12.0	.126	49.0
5	2-1				1			50.5	51.5	52.5	32.0	25.1	31.0	23.0	.126	49.0
11	2-1				1		1	48.9	48.5	49.0	30.7	24.7	26.5	21.5	.126	49.0
												21.2	27.0	17.0	.121	93.9

OUR OSURES

Num-ber	A cla	Age clas.	Age class	M	Mini-mum	M m	Soil tem-perature	Pcecnu	Maxi-mum	M m	Gram. calories	Foot-candles
4	2-1	2-1	1	56.5	55.5	57.0		18.4	21.5	6.0	0.45	0
4	2-1	2-1	1	55.9	55.0	56.0	40.0	20.6	23.0	9.0	.45	0
4	1-1	1-1	1	55.8	54.5	56.5	44.2	19.9	23.0	5.0	.45	0
4	2-1	2-1	1	55.0	54.5	55.5	37.0	17.2	23.0	4.0	.45	0
4	2-1	2-1	1	54.8	53.0	57.0	49.0	17.1	22.0	7.0	.45	0
4	2-1	2-1	1	54.2	52.5	54.5	39.0	18.3	20.5	3.0	.45	0
4	2-1	2-1	1	54.0	54.0	54.0	46.4	15.0	22.5	3.0	.45	0
4	2-1	2-1	0	53.9	53.5	53.5	46.0	18.7	22.5	5.5	.45	0
4	2-1	2-1	1	53.8	52.0	53.0	42.5	16.4	19.7	7.5	.45	0
4	1-1	1-1	0	52.5	52.0	52.5		16.1	19.5	5.5	.45	0
5	2-1	2-1	1	52.0	52.0	53.0	43.1	20.0	24.5	6.0	.45	0
4	2-1	2-1	1	52.0	51.5	52.0	43.4	19.6	23.5	2.0	.45	0
4	1-1	1-1	1	51.9	50.0	53.0	45.4	18.7	22.7	4.0	.45	0
4	2-1	2-1	1	51.8	50.5	52.5	46.5	15.8	25.5	7.5	.45	0
4	2-1	2-1	1	51.0	50.5	51.5	45.7	20.4	22.5	6.9	.45	0
10	2-1	2-1	1	50.0	49.0	51.0	36.0	18.8	21.0	17.0	.45	0

EXPERIMENTAL DATA

THE WATER BATH

Submerging the tops of plants in hot water resulted in no injury to roots, nor did submergence of roots result in injury to tops. However, after a week or more, plants which had either top or root killed by the exposure died completely. The length of the period during which one part was able to survive with the other dead was greater in older plants.

TABLE 4.—*Results of exposing tops, roots, or entire young conifers of different age classes to hot water for various lengths of time*

LOWEST KILLING TEMPERATURE [1]

Length of exposure and parts submerged	Part of plant	Norway pine			White pine			Jack pine	White spruce
		1-0	1-1	1-2	1-0	1-1	1-2	1-0	1-0
2 hours:		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
	Buds	(48.2)	(48.2)	48.9	(48.2)	(48.2)	48.9	48.2	(48.4)
Tops only	Needles	(48.2)	(48.2)	51.3	(48.2)	(48.2)	51.3	48.2	(48.4)
	Stems	(48.2)	(48.2)	51.3	(48.2)	(48.2)	51.3	48.2	(48.4)
Roots only	Roots	45.7	48.2	50.3	45.7	48.2	50.3	45.7	44.3
	Buds	46.9	48.2	50.3	46.9	48.2	50.3	45.7	45.9
Entire plant	Needles	46.9	48.2	50.3	46.9	48.2	50.3	45.7	45.9
	Stems	46.9	48.2	51.7	46.9	48.2	51.3	45.7	45.9
	Roots	46.9	45.7	50.3	45.7	45.7	50.3	45.7	44.3
5 hours:									
	Buds	48.4	48.4	--------	(48.4)	48.4	--------	45.9	(48.4)
Tops only	Needles	48.4	48.4	--------	(48.4)	48.4	--------	45.9	(48.4)
	Stems	48.4	(48.4)	--------	(48.4)	(48.4)	--------	45.9	(48.4)
Roots only	Roots	45.9	44.3	--------	44.3	45.9	--------	44.3	44.3
	Buds	44.3	45.9	--------	44.3	45.9	--------	44.3	44.3
Entire plant	Needles	44.3	45.9	--------	44.3	45.9	--------	44.3	44.3
	Stems	44.3	45.9	--------	44.3	(48.4)	--------	44.3	44.3
	Roots	44.3	45.9	--------	44.3	45.9	--------	44.3	44.3

LOWEST TEMPERATURE CAUSING INJURY [2]

Length of exposure and parts submerged	Part of plant	Norway pine			White pine			Jack pine	White spruce
2 hours:									
	Buds	47.1	(48.2)	47.6	(48.2)	(48.2)	46.9	47.1	48.4
Tops only	Needles	46.9	45.7	47.1	(48.2)	(48.2)	46.9	46.9	48.4
	Stems	47.1	(48.2)	48.9	(48.2)	(48.2)	48.9	47.1	48.4
Roots only	Roots	45.7	45.7	46.9	45.7	45.7	46.9	45.7	44.3
	Buds	45.7	45.7	46.9	46.9	45.7	47.1	45.7	44.3
Entire plant	Needles	45.7	45.7	46.9	46.9	45.7	47.1	45.7	44.3
	Stems	45.7	45.7	46.9	46.9	45.7	47.6	45.7	44.3
	Roots	45.7	45.7	46.9	45.7	45.7	46.9	45.7	44.3
5 hours:									
	Buds	44.3	44.3		48.4	45.9		44.3	48.4
Tops only	Needles	44.3	44.3		48.4	44.3		44.3	48.4
	Stems	44.3	48.4		48.4	48.4		44.3	48.4
Roots only	Roots	44.3	44.3		44.3	44.3		44.3	44.3
	Buds	44.3	44.3		44.3	44.3		44.3	44.3
Entire plant	Needles	44.3	44.3		44.3	44.3		44.3	44.3
	Stems	44.3	45.9		44.3	45.9		44.3	44.3
	Roots	44.3	44.3		44.3	44.3		44.3	44.3

[1] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause death. Figures in italics are lowest temperatures used, and therefore may not be the lowest that would cause death.

[2] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause injury. Figures in italics are lowest temperatures used, and therefore may not be the lowest that would cause injury.

Complete data on all tests were summarized in large tables with symbols designating the degree of injury. These tables were used to analyze the results, and the conclusions presented were drawn directly from them. The tables presented in the text are made up of the more important values extracted from these general tables. While the

major points are clearly illustrated in the tabular material presented, unfortunately some of the minor points are less distinct than in the complete data.

The critical killing temperatures, as determined by these tests, are given in table 4. The values may be referred to table 1 to determine the highest temperature which did not kill. For instance, the killing temperature with 2-hour top exposure for needles of 1–2 Norway pine, is, from table 4, 51.3° C. The next lower temperature used (table 1) was 50.3°, which failed to kill the needles completely.

An examination of table 4 reveals certain fairly definite trends regarding the resistance of different species and age classes to high temperatures. As would be expected, 5-hour exposures caused killing at lower temperatures than 2-hour exposures.

Between Norway and white pine there appears to be practically no difference in killing temperature. Spruce, when completely submerged, was killed at a temperature about 1° C. lower than Norway and white pine of the same age, while jack pine was the most tender of all. In resisting injuries due to high temperature, both white pine and white spruce appear to have a slight advantage over Norway pine and jack pine, whether the exposure be of 2-hour or 5-hour duration. The older age classes were able to survive higher temperatures than the younger ones quite irrespective of exposure time. However, greater age did not change susceptibility to injury. In every case the first parts to show injury were the fibrous roots, younger needles, and tender new stems. Injury was related to age of tissue or organ rather than to total age of plant. This was particularly evident when the plants were examined. If completely submerged, the tender fibrous roots were killed first, followed by the younger needles and new stem growth. Further increases in temperature injured the older needles and heavier roots, and finally the stem. The portion above the root collar, which is most massive and best protected by insulating bark, was with all plants the last to be killed.

Complete submergence resulted in injury and death to tops at lower temperatures than when only the tops were exposed to the hot water. In only 1 case out of 24 did a temperature as low as 45.7° C. for 2 hours cause important injury to tops when only this part was exposed, but when the entire plant was exposed to this temperature injury occurred in 14 cases. This may have been due to the greater physiological shock produced by killing of large amounts of tissue. It has been shown that dead root systems may supply the top of a plant with water for several days after death (*11*). Undoubtedly toxic substances from decaying roots would be swept along in the transpiration stream, adding to the injury already caused by heat.

EXPOSURE TO MOIST HEAT

The average killing temperature for all species exposed for 5 hours to hot air of high relative humidity was about 51° C. (table 5). These tests revealed no significant differences among either species or age classes. The needles were killed at lower temperatures than the buds and stems, and new growth was killed at lower temperatures than old growth.

TABLE 5.—*Effects of high temperatures in moist air on young conifers of different age classes*

LOWEST KILLING TEMPERATURE[1]

Length of exposure and plant part	Norway pine		White pine		Jack pine		White spruce	
	2-1-1	1-2	2-1-1	1-2	1-2	1-1	1-2	1-1
2 hours:	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Buds		58.1		58.1				
Needles		52.0		52.0				
Stems		58.1		58.1				
5 hours:								
Buds	*51.4*	51.6	52.1	51.6	51.4	(48.9)	51.4	(48.9)
Needles	*51.4*	49.9	51.4	49.6	51.4	(48.9)	51.4	(48.9)
Stems	*51.4*	51.6	52.6	51.6	52.1	48.9	51.4	(48.9)

LOWEST TEMPERATURE CAUSING INJURY[2]

2 hours:								
Buds		50.2		52.0			(45.6)	
Needles		50.2		50.2			(45.6)	
Stems		50.2		52.0			(45.6)	
5 hours:								
Buds		47.8	51.4		47.9	(48.9)	*47.9*	*47.8*
Needles		*47.8*	47.8		*47.9*	*47.8*	*47.9*	*47.8*
Stems		48.9	51.4		*47.9*	(48.9)	48.1	(48.9)

[1] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause death. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause death.

[2] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause injury. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause injury.

The killing temperature in moist air with 5 hours' exposure seems to be somewhat lower than the killing temperature in water with 2 hours' exposure or less.

Temperatures causing injuries to the plants are also shown in table 5. The differences among species and age classes are greater in regard to injurious temperatures than to lethal temperatures. This was undoubtedly due in part to the uneven stages in vegetative development of the plants tested. An old plant with tender needles will show injury at about the same temperature as a newly germinated seedling but death of the entire plant does not occur until much higher values are attained. Again, the stem and bud were usually more resistant than the needles. The younger age classes appear to have the advantage in their resistance to injury at high temperatures. This is probably due in large part to the greater height of older plants and the consequently greater radiation intensity to which they were exposed. Spruce was least resistant to injury and white pine most resistant. Jack pine appears to be decidedly more resistant than Norway pine.

Resistance to injury at high temperatures is not correlated with recovery from injury. It was found that in this respect Norway pine is the poorest of the lot and white pine and white spruce the best. When Norway pine has all its needles and buds killed, the plant usually succumbs even though the stem may be little injured. White pine and white spruce, on the other hand, will send out new buds from the heavier portion of the stem and eventually recover. Jack pine more closely resembles Norway pine in its inability to recover from injuries due to high temperatures.

EXPOSURE TO DRY HEAT

The significant results of the tests of effect on conifers of air of high temperature and low relative humidity are given in table 6. The killing temperature for 2-hour exposures in dry air for needles of the larger plants was 54° and for stems 57.7° C. For 5-hour exposures the killing temperatures varied from 52° C. for needles to over 56.5° for stems. These values are significantly higher than the corresponding ones for the tests in moist air. The lowest temperatures causing injury were also about 3° to 5° higher than those in moist air. Again the stems were most resistant to high temperatures and the needles least so. Except for the 1–0 jack pine, which was particularly tender, there seems to be little difference due to age class, and this difference is probably due more to the stage of vegetative development, to the greater radiation intensities endured by the taller plants, and to the greater drought stress of larger plants, than to any inherent changes in resistance with age beyond the juvenile stages.

The differences due to species were also slight, but because of their preponderance in one direction they appear to be important. During the examination the species were listed in the order of greatest injury. Out of 30 tests of Norway pine and white pine, the former received the greater injury 24 times. There was no correlation between age class and susceptibility of Norway pine to greatest injury.

TABLE 6.—*Effects of high temperatures in dry air on young conifers of different age classes*

LOWEST KILLING TEMPERATURE [1]

Length of exposure and plant part	Norway pine			White pine		Jack pine		White spruce
	2-1-1	1-2	1-1	2-1-1	1-1	1-1	1-0	1-1
2 hours:	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Buds	54.8	(53.7)	--------	54.8	--------	(54.3)	--------	(54.3)
Needles	54.0	(53.7)	--------	54.8	--------	(54.3)	--------	(54.3)
Stems	57.7	(53.7)	--------	57.7	--------	(54.3)	--------	(54.3)
5 hours:								
Buds	56.5	--------	54.8	54.8	(55.8)	54.8	53.0	54.8
Needles	53.9	--------	52.0	54.8	55.8	54.8	53.0	54.8
Stems	(56.5)	--------	(54.8)	(56.5)	(55.8)	(56.5)	53.0	(56.5)

LOWEST TEMPERATURE CAUSING INJURY [2]

	2-1-1	1-2	1-1	2-1-1	1-1	1-1	1-0	1-1
2 hours:								
Buds	50.2	53.7	--------	53.7	53.3	52.7	--------	52.7
Needles	48.9	51.5	--------	48.9	52.7	50.2	--------	50.2
Stems	54.8	53.7	--------	54.8	(53.3)	(54.3)	--------	(54.3)
5 hours:								
Buds	52.0	--------	52.0	52.0	55.8	51.0	--------	52.0
Needles	50.0	--------	51.8	51.0	52.0	51.0	--------	50.0
Stems	53.9	--------	54.8	53.9	55.8	54.0	--------	54.0

[1] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause death. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause death.

[2] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause injury. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause injury.

All four species were represented in 24 test runs. In these, Norway pine received greatest injury 15 times, white pine 4 times, jack pine 3 times, and white spruce twice. Rating the species according to

relative susceptibility to injury, with the highest score representing the greatest susceptibility, Norway pine from the 24 tests had a total score of 81, white pine 64, jack pine 49, and white spruce 46. This would indicate that Norway pine is in reality most susceptible to heat injury in dry air, with jack pine and white spruce distinctly more resistant, and white pine intermediate.

The influence of light intensity upon killing temperatures at low relative humidities was studied by comparing tests 34 and 35 in darkness with test 36, which had 306 footcandles illumination. The results of these tests were examined carefully to determine whether there were any significant differences due to light intensity. Since other conditions were essentially the same for the three exposures, any difference due to light as such should be apparent. The plants from the three tests were grouped by species and the order of injury determined for each. The number "1" was assigned to the plant receiving least injury, "2" to the next most resistant, and so on. The average scores for the plants in light and those in darkness are given in table 7.

TABLE 7.—*Comparative resistance of young conifers when exposed to the same temperature in light and in darkness*

Species	Mean score	
	Light	Dark
Norway pine	7.0	6.3
White pine	4.5	7.5
Jack pine	7.7	5.9
White spruce	6.7	6.3

Except for white pine there is no significant difference, and even here the difference is little greater than might be expected from chance variation. If light, through its influence on transpiration (*1*), has any important effect upon killing temperature, it was not disclosed by these tests.

DISCUSSION OF RESULTS

For determining the true resistance of conifers to high temperature, the tests in hot water were best, since it may safely be assumed that the parts submerged rather quickly attained the temperature of the water bath. For tests in air, owing both to transpiration, which has been shown to be important even at 88 percent relative humidity (*1*), and to the natural insulation provided by bud scales and bark, the plant parts were probably at a temperature of 2° to 3° C. below that of the surrounding air. Assuming that the killing temperature for the tissue is actually the same in water and air, we may compute the possible temperature difference which prevailed between plant parts and surrounding air. Such values are given in table 8 for 5-hour exposures of Norway pine and white pine. In moist air the killing temperature for buds was at least 3° and for needles 2° above that in water. In dry air the increases in temperature required to kill were more than twice as great except in the case of Norway pine needles.

TABLE 8.—*Increases in external killing temperatures for buds and needles of young Norway and white pines in air as compared with those in water, for 5-hour exposures*

| Species | Plant part | Temperature increase | |
		In moist air	In dry air
		°C.	°C.
Norway pine	Buds	3.1	6.4
	Needles	2.3	3.6
White pine	Buds	3.5	7.4
	Needles	2.1	7.4

Two factors operate to keep the plant temperature below that of air temperature: (1) Transpiration, and (2) the fact that the plant temperature was below air temperature at the beginning of the test. The greater the specific heat and heat conductivity of the medium in which the plants are exposed, the more quickly will they attain the temperature of the medium, other factors being equal. Consequently, the plants were heated most rapidly in water, and more rapidly in moist air than in dry air. However, in view of the length of the exposures, the rapid circulation of air, and the fact that the plants were also exposed to radiation which would be more effective in dry air than in moist air, it is believed that the more rapid rate of heating, at least in moist air, had little influence on the killing temperature of needles. The dominant factor must have been transpiration. While no especial effort was made to study this specific question in detail, the writer submits the method used as one more likely to give a positive answer to this question than studies in which the high temperatures are produced by excessive radiation alone, or studies of thermal emissivity.

Whether moisture in the air would play any important role in determining the killing temperature for conifers when the soil is dry was not determined. Under such conditions transpiration would be considerably reduced, and any cooling value it might have would be lessened. It would be difficult to determine the influence of humidity on killing temperature of plants in dry soil, since in tests as long as 5 hours in duration desiccation might be a more important factor than heat.

Another important factor in determining the highest temperature a plant will withstand is the duration of the temperature. Referring to tables 4 and 5, it is seen that a 5-hour exposure results in death at considerably lower temperatures than a 2-hour exposure. Such a result might be expected and has been reported by other investigators.

The fact that in the submergence tests the needles and buds were about equally resistant to high temperatures while in the tests in air the buds were more resistant probably means that because of superior insulation and lighter color the buds were not heated to as high temperatures as the needles. The difference in killing temperatures in moist and dry air for buds was less than that for needles. Likewise, in the more massive portions of the plant, the live tissue is protected by light-colored insulating bark, which may have prevented overheating.

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That total radiation intensity had a direct effect on the killing temperature is shown by the fact that for plants with dense needles the upper, more exposed ones suffered much greater injury than the lower, shaded ones. In this respect there seemed to be no difference in effect between radiation provided by electric lamps and by electric heaters. At least, the killing temperature in dry air was not appreciably influenced by an addition of radiation within the visible region of the spectrum.

The relative ability of different species to withstand high temperatures is of particular interest. Norway pine, because of its greater resistance to fire and because both it and jack pine grow in hot, dry, exposed places, is ordinarily assumed to be more resistant to excessive heat than the other species, and white spruce is assumed to be least so. While the evidence from these tests is not complete enough to be unmistakably convincing, it indicates that Norway pine is least resistant during the first few years of life and white spruce fully as resistant as any of the other species that were tested.

From the standpoint of the forester and ecologist, one very significant thing shown by the tests is the comparative uniformity of killing temperatures for all four species. This indicates that when excessively high temperatures occur, all of these species are likely to suffer to about the same degree. Under natural conditions, when the temperature is high enough to kill one species, it will probably kill the other three. In choosing which species to plant on exposed sites where high temperatures are likely to occur, equal consideration may be given to all four species, insofar as this factor alone is concerned. Spruce may be expected to survive as well as jack pine or Norway pine. This statement does not, however, apply to drought resistance.

Still more important from the ecological and silvicultural standpoint than the actual killing temperature is the capacity of a plant to recover from severe injury due to high temperature. In this respect Norway pine is again the poorest and white pine the best. The ability to recover depends upon the capacity of the stem—which for all species is most resistant to heat—to send out epicormic sprouts. White pine appears to excel all others in this respect, followed by white spruce, while jack pine shows little tendency to epicormic sprouting and Norway pine practically none. Epicormic sprouting is valuable also in protecting the plants against injuries caused by browsing or breakage.

CONCLUSIONS

From tests of Norway pine, white pine, jack pine, and white spruce 1 to 4 years old for killing temperatures in a water bath, it may be concluded that: (1) Resistance to excessive heat increases with increasing age and increasing size or mass of plant and tissue; (2) tops are more resistant than roots; (3) with 2-hour exposures the maximum temperature that needles can withstand is 49° C.; (4) temperatures as low as 44.3° do not cause severe damage to tops in exposures up to 5 hours' duration, but may result in death to roots; (5) there is little difference among the species tested in their ability to withstand heat.

For comparable plant material the external killing temperature was higher in air than in water and higher in dry air than in moist air.

The maximum temperature which needles withstood for 5 hours' exposure in moist air (relative humidity, 85 percent) was 50° C., while in dry air (relative humidity, 15 percent) they withstood 54°. Exposures of 5 hours in dry air caused little injury at temperatures of 50°.

Resistance to injury in dry air appeared not to be influenced by radiation intensity in the visible region of the spectrum under the conditions prevailing in these tests.

The cooling effect of transpiration was probably the most important factor involved in the greater resistance of plants to heat in dry air.

Jack pine was least resistant to heat in water and Norway pine least resistant in dry air. In neither case were the differences among species of much significance.

Recovery from excessive heat injury was associated with the capacity to send out epicormic shoots from the uninjured stem. White pine and white spruce excelled in this respect, while jack pine and Norway pine were decidedly inferior. From the ecological stand-point this appears to be the only important difference among the species tested.

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DEVELOPMENT–TEMPERATURE CORRELATION IN THE GREEN BUG, TOXOPTERA GRAMINUM [1]

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INTRODUCTION

The rate of development of animals in relation to temperature has received much attention from biologists. When the time required for development is plotted against temperature, it is found that, in general, the period becomes shorter with higher temperature up to near the maximum of possible development, the curve approximating a hyperbola. When, instead of time, the inverse of time is used, an estimate of rate of development is obtained. This is comparable with rate of reproduction or of any other biological process. When this is plotted, with temperature units on the horizontal axis and rate units on the vertical axis, it will be seen that rate increases with temperature until the temperature is unfavorably high. From the lowest temperature allowing development, the rate line seems to rise with increasing steepness to more favorable temperatures, and then to rise as a straight line through most of the range, almost to the upper limit of development. At higher temperatures the line quickly turns downward.

Much of the work on this subject has been summarized by Ludwig (*8*).[2] Most physiologists have leaned toward a curve of gradually increasing steepness for representing the development-temperature relation. The exponential curve of Van't Hoff (*6*) and the modified curve of Arrhenius (*1*) have been much used. These curves fit the increase in rates of many chemical reactions with rising temperature, but in biology they seem to give better results with such processes as digestion and respiration than with development. Krogh (*7*), however, believed that the development-temperature curve was practically a straight line except at the extreme ends.

Entomologists have favored the expression of time-temperature as a hyperbola (Sanderson (*10*)) or of rate-temperature as a straight line (Sanderson and Peairs (*11*); Peairs (*9*); Chapman (*3, pp. 45–64*)). Bliss (*2*), however, used Arrhenius' curve. Shelford (*12, p. 180*) suggested that a straight line could be used in the "medial" range, but not at the extremes.

EXPERIMENTAL PROCEDURE

This paper presents an analysis of data which were obtained at the Minnesota Agricultural Experiment Station in 1927 in a study of the green bug (*Toxoptera graminum* Rond.). The rearing has been described by the writer (*13, p. 330*), and the temperature cabinets used have been described by Chapman (*3, p. 41*). The thermostatic

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control was not then so well developed as with the equipment described, but was satisfactory and yielded comparable results. Groups of these aphids were reared from birth to maturity at constant temperatures, ranging from 7° to 33° C. A total of 404 wingless individuals were reared. The time required ranged from 5 to 37 days, depending largely on the temperature.

The time for each individual was expressed as a whole number of days; while precision might have been improved by using records in fractions of days, general tendencies are well shown. Rate was expressed in thousandths of total development per day; thus, an aphid requiring 30 days to develop had a rate of development expressed as 33.3, while one requiring 8 days had a rate expressed as 125.0. Either all the individual cases or the means of groups reared at each temperature, when plotted, give the usual hyperbola form for time-temperature. The curve for rate-temperature is also the typical one, with the slightly flattened threshold, the nearly straight line through a large part of the range, and lessening slope and quick downturn toward the upper limit. Data on time and rate of development are given in table 1.

TABLE 1.—*Time and rate of development of Toxoptera graminum, by individual cases, at various constant temperatures*

Time	Rate (1,000 ÷ days)	Number of individuals reared at—											
		7° C.	10° C.	12° C.	14° C.	17.5° C.	20° C.	22° C.	23° C.	26° C.	30° C.	32° C.	33° C.
37 days	27.0		2										
36 days	27.8	2	1										
25 days	28.6	0	5										
34 days	29.4	0	3										
33 days	30.3	0	5										
32 days	31.2	0	7										
31 days	32.3	1	8										
30 days	33.3	1	4										
29 days	34.5		4										
28 days	35.7		3										
27 days	37.0		1	1									
26 days	38.5		1	1									
25 days	40.0			0									
24 days	41.7			0									
23 days	43.5			1									
22 days	45.5			1	1								
21 days	47.6			2	2								
20 days	50.0			11	5								
19 days	52.6			4	5								
18 days	55.6				19								
17 days	58.8				10								
16 days	62.5				4								
15 days	66.7				2								
14 days	71.4				1	1						1	
13 days	76.9					2						0	
12 days	83.3					21						0	
11 days	90.9					19						0	
10 days	100.0					9	4					0	
9 days	111.1					1	20	1	1			1	
8 days	125.0						22	6	8		4	2	
7 days	142.9						5	14	23	18	5	3	2
6 days	166.7							2	6	12	41	10	
5 days	200.0									1	21		
Total		4	44	21	49	53	51	23	38	31	71	17	2

At the same time there were reared 103 specimens of the winged form, which is found with the wingless in many families of this species. These were not used in the analysis, as they differed slightly from the wingless forms, and their smaller numbers made a separate study

undesirable. Their rate-temperature curve was of the same form as that of their wingless sisters, but of slightly different slope; at most temperatures the time of development differed significantly from the time required by the wingless form. The rate of reproduction (*13*, *p. 333*) showed a curve of the same general form, but with more marked curvature, a peak at a lower temperature (about 22° C.), and a longer declining phase. These data were not suited in numbers and arrangement for statistical study.

EXPERIMENTAL RESULTS

Above 33° C. nymphs failed to mature, although some lived for several days. A few signs of development were shown a little below 7°, but difficulty of rearing at this temperature made it seem unlikely that any would mature at lower temperatures. At 7° only 4 were successfully reared; many died during development. At 33° only 2 were reared. At 32°, 17 were reared after a number of trials, but many failed to mature and others were retarded considerably in development, generation rearing was difficult, and decreased vigor was shown in several ways. At each of these three temperatures only a minority of those started were reared, while at the intervening temperatures all or nearly all matured. The few maturing at the extreme temperatures may have been a selected group, and the data therefore seem unsafe, both statistically and physiologically, to use in a critical study of the curve of developmental rate and temperature.

The remaining individuals, 381 in all, were reared at nine constant temperatures from 10° to 30° C. The results seemed to be quite normal; of the nymphs started, a large majority matured. While at 30° adults showed depressing effects of high temperatures in shortened life, increase was rapid and generation rearing not difficult. Those reared in this range of temperatures were accordingly studied to determine the best temperature-development curve, the criterion being the standard error of estimate as defined by Ezekiel (*4*). By dropping the few extreme cases the flattening at the threshold and the downturn at the upper end are eliminated, and a nearly straight line is obtained, with slight increase in steepness in the lower part and a slight decrease in the upper part.

STATISTICAL ANALYSIS

The curve of increasing steepness worked out by Arrhenius (*1*) was tried first. The preliminary test of fit for this curve is the plotting of the logarithm of the rate on the horizontal axis and the reciprocal of the absolute temperature on the vertical. If the curve is adapted to the data, this plotting should give a straight line sloping upward from left to right. The means were used in this test, and the plotting indicated that the curve was not adapted to the material. The line showed a definite bend in the middle, but even when divided at the bend the resulting two lines did not promise a good fit. The "Q 10" curve of Van't Hoff seemed even less likely to fit.

The next test was with a logarithmic curve (fig. 1). It is well adapted to gently curving lines, and can be fitted by least squares in the same manner as a straight line, except that the logarithms of one or both variables are used instead of the absolute values. The logarithms may be plotted as a preliminary test, a straight line indi-

cating a probable fit. In this case the logarithms of the mean rates were plotted against the arithmetical values of the temperatures, and vice versa, and a third plotting of the logarithms of both quantities was made. All these test plottings showed the tendency, already noted, to change in slope in the middle; none gave promise of markedly good fit, but the use of logarithms on both sides gave the best
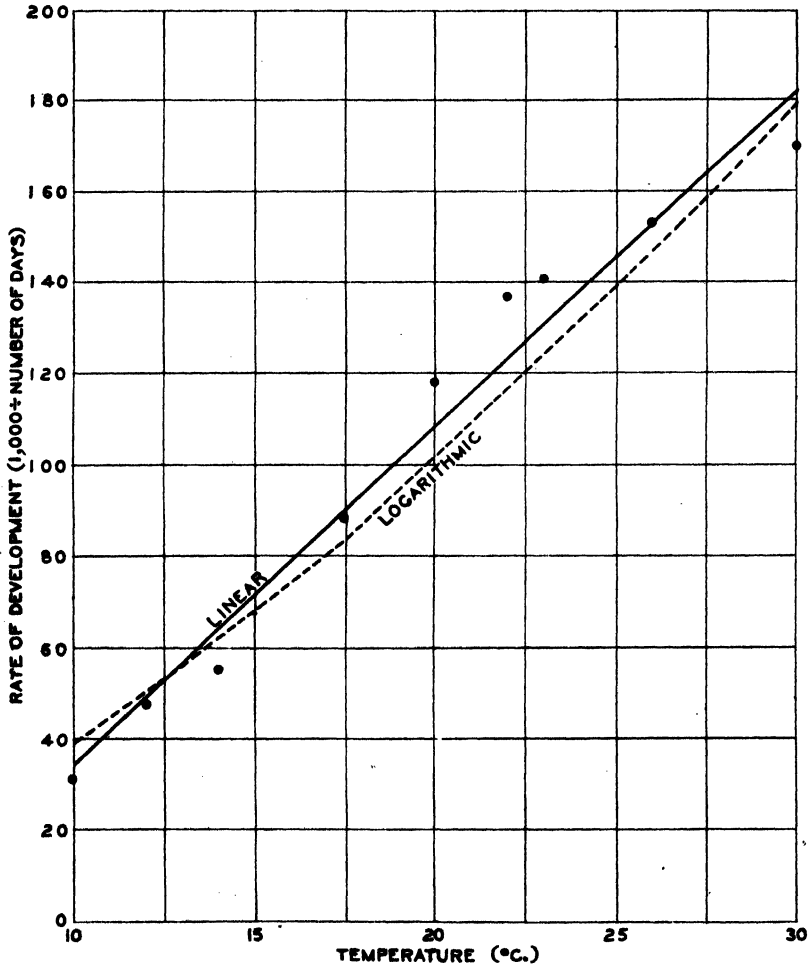
FIGURE 1.—Straight-line and logarithmic curves fitted to rate of development of *Toxoptera graminum*.

promise. The use of logarithms of rates against arithmetical temperature values gave a nearly straight line in the material reared at from 10° to 20° C. Accordingly, the double logarithmic curve was worked out by least squares from the individual cases,[3] and gave a fair fit with a nearly straight curve of very slightly increasing steepness. The formula was log $Y=0.200974+1.391$ log X, and the standard error of estimate 16.75 thousandths of total development per day.

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[3] For convenience, only means are shown in the various figures.

Next was fitted a freehand curve of a single bend, with slowly increasing steepness, according to the method described by Ezekiel (*3*). In this case the standard error of estimate was 18.94 thousandths of total development per day.

A straight line (fig. 1) was fitted by the method of least squares from individual cases, the formula being $Y = -38.07 + 7.34X$. A
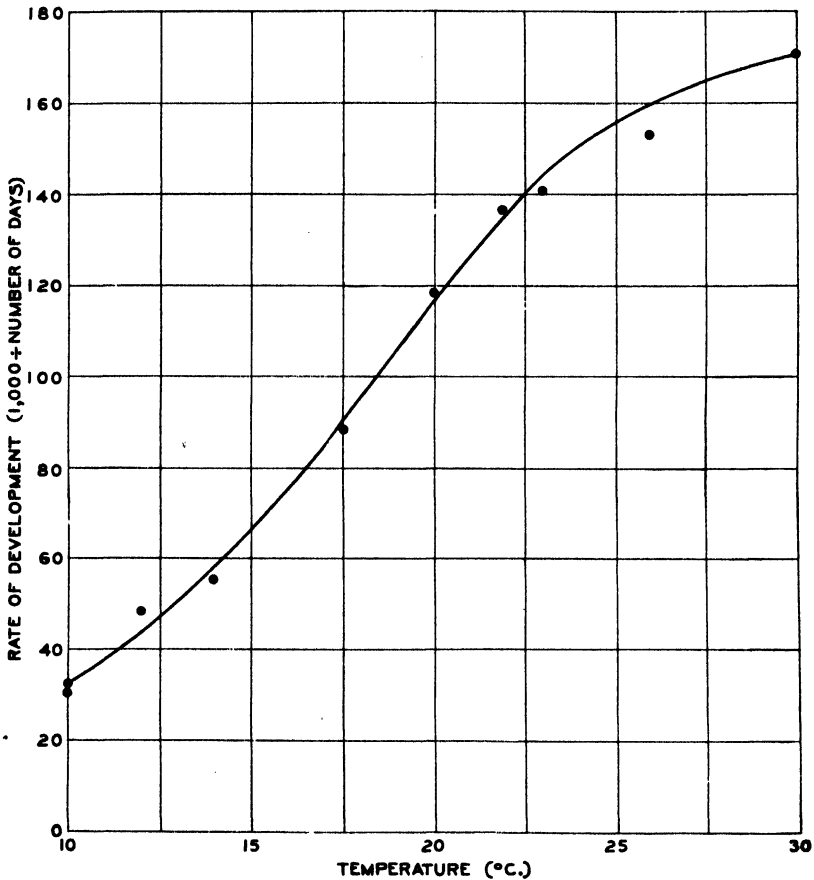
FIGURE 2.—Freehand S-curve fitted to rate of development of *Toxoptera graminum*.

better fit was obtained than with either of the other curves, the standard error of estimate being 14.56 thousandths of development per day.

A freehand curve (fig. 2) rising with increasing steepness to about 20° C., and then inflected to decreasing steepness, or in the form of a very elongated, tilted S, was then tried. This gave a standard error of estimate of 12.85 thousandths of total development per day, the lowest of any curve tried.

Errors of the standard errors of estimate given $\left(\dfrac{\text{S. E.}}{\sqrt{2n}} \right)$ are in

the neighborhood of 0.5 to 0.7, indicating differences significant or nearly so, the significance of the difference between the last two being

most doubtful. The freehand concave curve is significantly the poor-est, while the logarithmic curve, which is nearly straight, is a poorer fit than a straight line.

A straight line seems definitely better to represent this relation than single-bend curves of increasing steepness. It appears, however, that some curvature may be present. In view of the advantage the
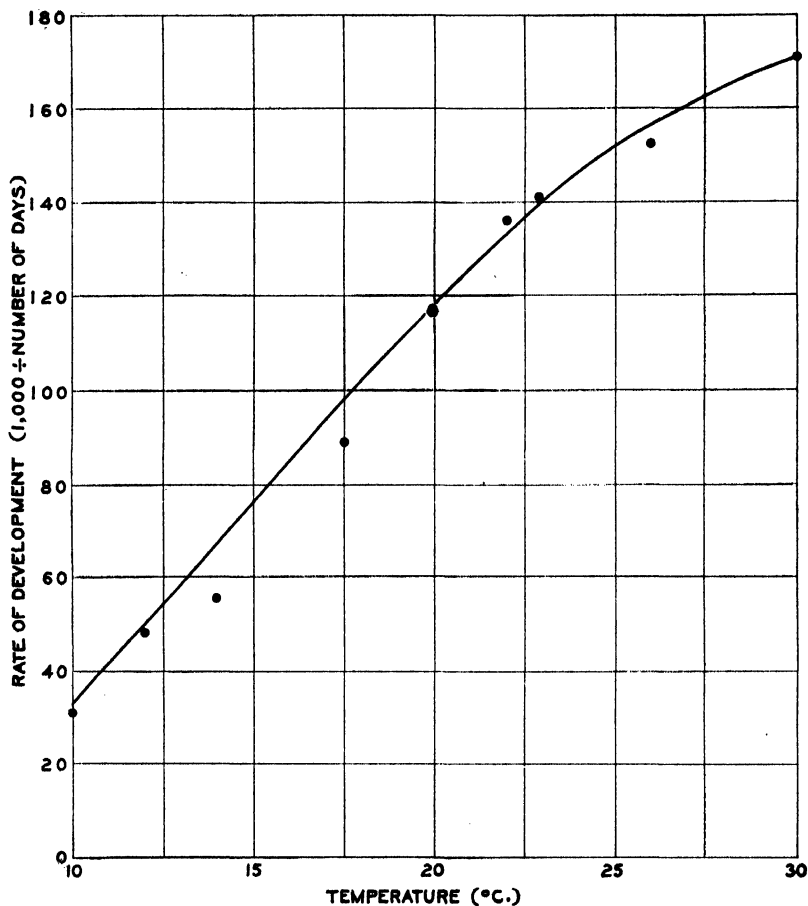
FIGURE 3.—Cubic parabola fitted to rate of development of *Toxoptera graminum*.

S-curve shows over a straight line, this possibility was further tested. When the material was divided and portions were studied separately, it was found that at from 10° to 20° C., inclusive, a concave curve was somewhat better than a straight line; while from 20° to 30° a convex curve was slightly better than a straight line. Blakeman's criterion of linearity was between 2.5 and 3.0. Both these studies of separate portions and Blakeman's test indicate, as did the comparisons of standard errors of estimate, that the advantage of the S-curve over the straight line approaches significance. Departure from linearity was tested by the method of Fisher (5, p. 216), and proved significant,

with a Z value of 1.25317 against a 1 percent point Z of 0.487. This is the strongest evidence found in favor of the S-curve, indicating that a definite variation not explained by linear regression or random variability exists. The S-curve seems best fitted to represent this condition.

Finally an effort was made to find a mathematical curve adapted to the material, since such a curve can be more easily described or reproduced than a hand-fitted curve. Several curves were found to fit fairly well within the limits of 10° and 30° C., although when extended outside the limits, they departed widely from the curve form usually associated with such material. One of these curves, a cubic parabola, was selected because it followed the general trend of biological material even beyond the limits. The correspondence was not so exact as to suggest a fundamental fitness, but in general this curve was the best. This parabola rose from 10° to 20° as nearly a straight line, with a slight tendency to curve at the lower end. Above 20° it ascended, with some decrease in slope, to a maximum near 30°. The formula was $Y = -32.6 + 3.74X + 0.373X^2 - 0.0091X^3$, and the standard error of estimate 13.85 thousandths of total development per day. The curve is illustrated in figure 3.

CONCLUSION

It is concluded that for the material used in this study a straight line is definitely better than a curve of increasing steepness for representing the development-temperature relation. Considerable evidence is found that an elongate S-curve may be better than a straight line. It is possible that, with more material and greater precision, the superiority of the S-curve would be better brought out. A freehand curve of this sort has given the best fit, but a segment of a cubic parabola has also given good results.

SUMMARY

A statistical analysis has been made of the rate of development of the green bug (*Toxoptera graminum* Rond.) in relation to temperature. Several curves have been fitted to developmental rate and temperature, and standard errors of estimate, in thousandths of total development per day, have been calculated.

Arrhenius' curve failed to show fitness. With the other curves the results are as follows: Double logarithmic curve, 16.75; freehand concave curve, 18.94; straight line, 14.56; freehand S-curve, 12.85. Futher statistical tests indicate the likelihood of some curvature. After a study of several mathematical curves, a cubic parabola, which gave a standard error of estimate of 13.85, was chosen. The straight line is a better expression of the development-temperature correlation in this material than concave curves, but considerable evidence is found that an S-shaped curve may be still better.

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THE CALCIUM AND INORGANIC PHOSPHORUS CON-
TENT OF THE BLOOD SERUM OF SWINE[1]

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INTRODUCTION

The object of this study was to ascertain the normal content of calcium and inorganic phosphorus in the blood serum of swine at various ages, and to determine the effect of a high and a low calcium diet on the amount of these elements. Bohstedt and his associates (*3*) [2] noted differences in these constituents in the blood of pigs when the calcium : phosphorus ratio varied greatly in the rations fed. Loeffel et al. (*14*) found wide differences between the maximum and minimum content of these two elements in the blood of the pig, and stated that the blood plasma of animals receiving the direct rays of the sun were higher in these elements than those maintained in the absence of direct sunlight. Hughes and Hart (*10*) reported an average content in the blood serum of swine of 11.77 mg of calcium and 8.53 mg of inorganic phosphorus in 103 samples taken at random in the California Experiment Station herd. Bethke, Edgington, and Kick (*2*) showed the serum calcium and inorganic phosphorus of pigs about 200 days of age to be 11.1 and 6.4 mg, respectively (average of lots 4, 5, 6, 7, 8, 9, and 10 of the third experiment). The average of some of the figures reported by Dunlop (*6*) are serum calcium, 11.32 mg and inorganic phosphorus in whole blood, 6.93 mg (average of 56 determinations of blood taken from hogs weighing from 50 to 200 pounds, in experiment 4, groups 1, 2, 3, and 6). Howland and Kramer (*9*) collected, summarized, and published much of the early work up to and including that of 1921 on the calcium and inorganic phosphorus content of the serum of plasma of domestic animals. In this summary it was shown that three workers had reported, respectively, the calcium content to be 8.7, 9.6, and 9.7 mg and one had reported the inorganic phosphorus to be 2.3 mg, in 100 cc of the blood serum of the pig.

PROCEDURE

Blood for analysis was secured by cutting off the tip of the tail and collecting from 50 to 60 cc in a small Erlenmeyer flask. It was necessary to kill the very small pigs to obtain a sample large enough for analysis. The blood was allowed to clot, the serum poured off, and then centrifuged. In a few cases where the sample was taken before the pig was allowed to suckle, the blood would not clot; in such cases it was impossible to determine the phosphorus. (It was impossible to obtain a clear solution after the addition of trichloracetic acid.) The clear serum obtained after centrifuging was used for analysis. Serum calcium was determined by the Clark-Collip (*4*) modification of the Kramer-Tisdall method and the inorganic phosphorus was ascertained by the method of Fiske and Subbarow (*7*).

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[1] Received for publication May 12, 1935; issued October 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 278.

NORMAL SERUM CALCIUM AND INORGANIC PHOSPHORUS OF PIGS AT DIFFERENT AGES

Since the literature indicated that the serum calcium and inorganic phosphorus of the very young animals are greater than those of older and mature animals (an exception is found in the case of chickens as reported by Hughes, Titus, and Smits (*11*), it seemed advisable in attempting to determine the normal blood calcium and inorganic phosphorus of the pig to divide the life span into limited parts or units.

Examples of some of the diets fed, and the calcium and phosphorus content of 100 pounds of the feed mixture, are given in table 1.

TABLE 1.—*Ingredients of the various diets fed and their calcium and phosphorus content*

Composition of diet (pounds)	Calcium	Phosphorus
	Percent	*Percent*
Barley, 43; rice bran, 30; alfalfa, 20; tankage, 5; salt, 1; CaCO₃, 1	1. 58	0. 88
Barley, 88; tankage, 10; salt, 1; CaCO₃, 1	1. 07	. 63
Barley, 52; wheat middlings, 15; tankage, 10; linseed meal, 3; rice bran, 15; alfalfa, 5; salt, 1; CaCO₃, 1	1. 28	. 78
Barley, 58.5; wheat middlings, 25; wheat bran, 14; salt, 1; CaCO₃, 1.5	. 69	. 52
Barley, 98.5; casein, 1.5; salt, 1; CaCO₃, 1; cod-liver oil (5–10 cc daily)	. 50	. 39

All pigs had free access to direct sunlight, some had access to pasture, and some were fed in dry lot.

While the diets were not all optimum for calcium and phosphorus as suggested by Simmonds (*15*), Bethke et al. (*2*), and Dunlop (*6*), the ratio of calcium to phosphorus in each case was in favor of calcium. Except for the last diet listed, the calcium and phosphorus were greater than the 0.65 g of calcium and 0.40 g of phosphorus in 100 g of the food mixture suggested by Simmonds as optimal for the rat. It contained slightly less phosphorus than that suggested by Bethke and his coworkers (*2*), but, the amount of vitamin D was optimum.

The serum calcium of 258 samples and the inorganic phosphorus of 232 samples of blood taken from normal pigs of various ages are shown in tables 2 and 3. These data are arranged to show any differences in the mean values for each age group. One determination of calcium in the blood taken from a pig immediately after the umbilical cord was severed is not shown. The serum calcium in this case was 17.32 mg, the highest for any normal pig recorded in these studies.

The range of the calcium for any one age is less than for the inorganic phosphorus. This is shown in the tables and is substantiated by the standard deviation for the means. The average content of serum calcium in these studies was 11.93 mg, which is slightly higher than the figure reported previously by the writer and Hart (*10*). In that instance only the blood of older pigs was examined, which would account for the difference. The calcium content of the blood of very young pigs (birth to 15 days) is higher than that of older hogs. The mean calcium content for all hogs over 70 days of age (216 determinations) was 11.65 mg, with a standard deviation of 0.69±0.022. The mean value for pigs 210 to 365 days of age is slightly higher than for pigs younger or older. In attempting to find an explanation for this

it was noted that in several instances blood samples had been taken just previous to or soon after farrowing. An average of 11 determin-. ations of the serum calcium and inorganic phosphorus taken from sows at this stage were 12.94 and 7.62 mg, respectively. The calcium content of the blood is apparently higher and the inorganic phosphorus is lower just before and immediately after farrowing.

TABLE 2.—*Calcium content of the blood serum of normal pigs, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of calcium in 100 cc								Mean [1] calcium in 100 cc	Standard deviation[1]
	9–10	10–11	11–12	12–13	13–14	14–15	15–16	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
Birth to 15	0	0	0	0	5	9	6	20	14.44	0.62±0.066
15 to 70	0	0	6	12	4	0	0	22	12.40	.49± .050
70 to 120	1	5	30	10	1	0	0	47	11.64	.61± .043
120 to 210	1	7	38	12	0	0	0	58	11.57	.55± .036
210 to 365	1	3	28	15	3	0	0	50	11.85	.72± .045
365 to 730	0	6	19	8	2	0	0	35	11.72	.68± .055
730+	1	5	16	3	1	0	0	26	11.40	.66± .062
Total or average	4	26	137	60	16	9	6	258	11.93	.99± .029

[1] From original, not group data.

TABLE 3.—*Inorganic phosphorus content of the blood serum of normal pigs, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc								Mean [1] inorganic phosphorus in 100 cc	Standard deviation[1]
	5–6	6–7	7–8	8–9	9–10	10–11	11–12	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
Birth to 15	0	0	0	7	2	5	0	14	9.37	0.77±0.098
15 to 70	0	4	2	7	3	3	1	20	8.61	1.31± .140
70 to 120	1	6	6	13	14	4	0	44	8.51	1.18± .085
120 to 210	1	3	7	20	14	3	3	51	8.66	1.20± .075
210 to 365	1	4	17	19	4	2	0	47	8.05	.96± .067
365–730	1	11	6	7	4	1	0	30	7.63	1.15± .100
730+	0	2	14	7	2	1	0	26	7.96	.86± .080
Total or average	4	30	52	80	43	19	4	232	8.34	1.19± .035

[1] From original, not group data.

While the inorganic phosphorus varied considerably for any one age, and while the value for very young pigs (birth to 15 days) is higher than for older hogs, the differences in the means are not so great as for the serum calcium. The mean value for inorganic phosphorus without the very young pigs (birth to 15 days) was 8.27 mg. Whether the higher content of serum calcium and inorganic phosphorus in the very young animal is due to the amount of these elements ingested from the milk of the mother, or whether high concentrations are mobilized in utero for the early growth of the body

tissues is not clear. The serum calcium continues to be relatively high during the entire suckling period.

Although calcium in the blood of the mother just before and soon after parturition was higher than normal, the maternal blood was not so high either in this element or in phosphorus as that of the newborn pig.

After the pig has attained an age of about 70 days (weaning time) there seems to be very little change in its serum calcium throughout life (except for periods immediately before and after farrowing). The inorganic phosphorus, which is higher at birth and for a short time thereafter, gradually decreases until maturity.

EFFECT OF A HIGH AND LOW CALCIUM DIET ON SERUM CALCIUM AND INORGANIC PHOSPHORUS OF SWINE

EFFECT OF HIGH CALCIUM DIET

Bohstedt and his coworkers (*3*) showed that the addition of 2 percent $CaCO_3$ to a diet of white corn, wheat middlings, linseed meal, and salt resulted in a higher content of serum calcium in pig's blood than did the basal diet. Dunlop (*6*) reported increased calcium and decreased phosphorus in the serum of pigs fed rations containing 1.62 percent of calcium, with a calcium-phosphorus ratio of 1.0 to 0.34. Bethke et al. (*2*) found the inorganic phosphorus of swine as low as 4.5 mg in 100 cc of serum when the phosphorus in the ration was from 0.31 to 0.33 percent. Hjort (*8*) stated that calcium salts administered orally to dogs caused a marked increase in the serum calcium of normal animals. Kramer and Howland (*13*) showed that increasing the calcium in the diet of rats increased the serum calcium and depressed the phosphorus.

In the studies relating to the effect of a high calcium diet, two methods of administering the calcium were employed: (1) The pigs were fed diets to which calcium carbonate was added; (2) calcium salts in solution or in suspension were administered directly into the pig's stomach, by means of a funnel and rubber tube. The diet fed in the first series of high calcium tests consisted of barley, 55 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent, to which was added 5 percent of $CaCO_3$. The calcium content of this diet was 2.09 percent and the phosphorus 0.51 percent. The time that elapsed from the beginning of the high-calcium feeding to the taking of the first blood samples ranged from 4 hours to 7 days. A summary of the results is given in tables 4 and 5. A few of the extremely high calcium determinations are not included. These will be discussed briefly later.

The mean calcium content is nearly 3 mg higher and the inorganic phosphorus is about 2.5 mg lower than for normal pigs of the same age. None of the calcium determinations was below 12 mg and none of the inorganic phosphorus determinations was above 8 mg. There is a wide range in the values for serum calcium. Some of the higher ones were from pigs in which calcium salts were administered directly into the stomach.

TABLE 4.—*Calcium content of the blood serum of hogs fed a high calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of calcium in 100 cc									Mean[1] calcium in 100 cc	Standard deviation
	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	Total samples		
	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Num-ber	Milli-grams	
70 to 120	4	7	0	1	0	0	0	0	12	13.10	0.75±0.103
210 to 365	11	21	16	12	4	4	3	4	75	14.75	1.83± .101
Total or average	15	28	16	13	4	4	3	4	87	14.53	1.80± .092

[1] From original, not group data.

TABLE 5.—*Inorganic phosphorus content of the blood serum of hogs fed a high calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc							Mean[1] inorganic phosphorus in 100 cc	Standard deviation
	3-4	4-5	5-6	6-7	7-8	8-9	Total samples		
	Number	Number	Number	Number	Number	Number	Number	Milli-grams	
70 to 120	0	0	0	5	5	1	11	7.18	0.69±0.099
210 to 365	2	19	22	11	4	1	59	5.50	1.01± .063
Total or average	2	19	22	16	9	2	70	5.77	1.15± .066

[1] From original, not group data.

To determine the effect of suddenly increasing the calcium content of the ration, a group of six pigs 8 months of age were fed for a short time on a ration of barley, wheat middlings, wheat bran, and 1.5 percent of $CaCO_3$, and two pigs of the same age similarly bred were fed a normal diet and kept as controls. On April 30, 1932, the $CaCO_3$ of the experimental animals was increased to 5 percent. The calcium and phosphorus in the ration were, respectively, 2.09 and 0.51 percent. Blood samples were taken and analyzed, as shown in table 6.

A third series of experiments designed to show the effect of administering calcium salts directly into the stomach was carried out. Eight pigs about 3½ months of age were used. Some of them were weaned and some were not. In the morning of July 18, 1932, their average blood calcium and phosphorus were, respectively, 11.97 and 7.3 mg. At 4:30 in the afternoon, 25 g of $CaCO_3$ in 125 cc of water was administered to four of the pigs. The others were kept in the same pen as controls. At 8:30 a. m., July 19, blood samples were taken and examined. The average for the experimental animals was calcium, 31.68 mg, and phosphorus, 6.85 mg. The average for the control animals was calcium, 12.41 mg, and phosphorus, 7.98 mg. The blood serum calcium of the experimental animals ranged from 27.01 to 34.26 mg. None of the pigs was fed on July 19, the day of sampling. On July 20 at 8:30 a. m. another sample of blood was

taken. The average serum calcium of the experimental animals was 10.97 mg and that of the control animals 11.32 mg. During the 24-hour interim the excess serum calcium had disappeared from the blood.

TABLE 6.—*Calcium and inorganic phosphorus in 100 cc of serum of a group of pigs 8 months of age when the calcium in the diet was suddenly increased* [1]

Date of sampling	Samples taken	Calcium	Inorganic phosphorus	Date of sampling	Samples taken	Calcium	Inorganic phosphorus
	Number	*Milligrams*	*Milligrams*		*Number*	*Milligrams*	*Milligrams*
May 2_____	4	17.28	5.79	May 19_____	6	14.25	5.61
May 3_____	6	16.35	5.37	May 24_____	6	14.32	5.69
May 4_____	6	16.54	4.39	June 15_____	6	13.52	6.75
May 11_____	6	15.35	4.96				

[1] 2 controls fed a normal diet had an average of 12.40 mg calcium and 7.51 mg phosphorus per 100 cc serum for the same period.

The evidence as reported in table 4 shows definitely that the serum calcium of the pig can be increased by giving a large quantity of calcium in the diet. As measured by the calcium content of the serum, pigs show a rapid response either to feeding a high calcium diet, or to having calcium salts administered directly into the stomach. While the feeding of a high calcium diet causes an immediate rise in the serum calcium and while continuous feeding of such a diet results in the calcium remaining above normal, after a time the calcium appears to decrease slowly. Apparently the body attempts to return the serum calcium and inorganic phosphorus to normal values but is unable to accomplish this as long as a marked excess of calcium is furnished. Kramer and Howland (*13*) have postulated that a serious high concentration of calcium and phosphorus in the blood is avoided by excretion of the excess of the kidneys, the intestines, or by the deposition of part of these materials as insoluble calcium phosphate in the bones. A single dose of a calcium salt introduced indirectly into the stomach causes a sudden rise, which is followed by a relatively rapid fall in the serum calcium, extending over a period of not more than 24 hours.

That there is a reciprocal relation of the serum calcium and inorganic phosphorus of the blood of the pig under most conditions seems certain. Under normal conditions the blood calcium and inorganic phosphorus fluctuate round a certain mean; but as the calcium content of the diet is raised the serum calcium of the blood increases and at the same time the inorganic phosphorus decreases.

EFFECT OF LOW CALCIUM DIET

As early as 1920 Kramer and Howland (*12*) showed that the calcium of the blood serum of children affected with tetany was far below that of normal adults, or that of placental blood. Six years later Bohstedt et al. (*3*) reported that pigs fed diets containing less than 0.10 percent of calcium had lower blood serum calcium than those fed from 0.50 to 0.80 percent. In 1930 Loeffel and his coworkers (*14*) found that pigs fed yellow corn, soybean-oil meal, blood meal, dried skim milk, and salt, indoors, had lower blood serum calcium and

inorganic phosphorus than those fed the same ration outdoors. Hughes and Hart (*10*) in 1932 reported a decrease in the serum calcium of two sows fed a ration deficient in calcium.   Kramer and Howland (*13*) showed that when the phosphorus in the diet of rats was above normal (except when an adequate amount of calcium was fed to counteract the effect), or when the calcium concentration was reduced to a minimal value, there resulted a decrease in the calcium and an increase in the organic phosphorus in the serum of the rat. Benjamin and Hess (*1*) in tests with young rats on a high calcium diet, and Templin and Steenbock (*16*) in tests with mature rats on a low calcium diet, found that the serum calcium of the animals fed a basal diet was lower than that of animals fed the same diet to which had been added various antirachitic agents such as cod-liver oil, ultraviolet irradiation, and irradiated ergosterol.

The object of the experiments described below was to determine whether rations having an inadequate amount of calcium would effect a change in the calcium and inorganic phosphorus content of the blood serum of pigs of different ages, and whether the physical well-being of the experimental animals would be affected.   The rations fed were as follows:

    Barley, 99 percent; salt, 1 percent.
    Barley, 99 percent; sodium bicarbonate, 1 percent.
    Barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent.

The percentage of calcium and phosphorus in the first two diets was, respectively, 0.091 and 0.385, while the third ration contained 0.095 percent of calcium and 0.53 percent of phosphorus; the ratio of calcium to phosphorus being roughly 1:4.0 and 1:5.5.

TABLE 7.—*Calcium content of the blood serum of hogs fed a low calcium diet, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of calcium in 100 cc									Total samples	Mean[1] calcium in 100 cc	Standard deviation
	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
15 to 70			1	3	0	4	2	9	1	20	10.21	1.63±0.174
70 to 120	1	3	6	2	1	2	6	4	-----	25	8.47	2.17±.207
120 to 210				1	2	10	18	15	1	47	10.47	.87±.061
210 to 365			1	1	3	2	10	--		17	9.56	1.09±.125
365 to 730			4	4	3	0	4	7	-----	22	9.27	1.92±.196
730+	2	13	12	10	9	5	10	6	1	68	7.99	2.05±.118
Total or average	3	16	24	21	18	23	50	41	3	199	9.13	2.02±.068

[1] From original, not group data.

A summary of the findings in these tests is given in tables 7 and 8. The mean value for serum calcium of 199 determinations of blood serum of pigs of all ages was 9.13 mg; the mean of 182 determinations for inorganic phosphorus was 8.44 mg.   The value of calcium in this case was more than 2.50 mg less than the normal serum calcium of pigs of the same ages.   The serum calcium of 68 samples of

blood taken from animals 2 years old and older is considerably below the mean. This may be accounted for in part by the fact that several older animals were on a low calcium diet for a long period. Of particular interest is the fact that the inorganic phosphorus of young pigs fed a low calcium diet, some of which were still with their mothers, was much lower than that of older hogs, and was far below the normal for pigs of the same age (p. —). Likewise the inorganic phosphorus for hogs 2 years old or over was higher than the average, while the average of the serum calcium for the same individuals was lower.

TABLE 8.—*Inorganic phosphorus content of the blood serum of hogs fed a low calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc										Mean [1] inorganic phosphorus in 100 cc	Standard deviation
	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
15 to 70	2	3	8	3	1	1				18	6.54	1.21±0.136
70 to 120	1	2	7	5	4	1	1	1		22	7.45	1.60± .163
120 to 210			5	9	9	8	8	1		40	8.61	1.36± .103
210 to 365		1	1	3	5	5	1			16	8.51	1.19± .142
365 to 730		1	3	3	11	2	1	1		[2] 23	8.49	1.79± .178
730+			4	11	11	22	10	3	1	[3] 63	9.17	1.40± .084
Total or average	3	7	28	34	41	39	21	6	1	182	8.44	1.66± .059

[1] From original, not group data.
[2] 1 determination of 14.15 mg included but not shown in the table.
[3] 1 determination of 13.55 mg included but not shown in the table.

As would be expected, the serum calcium and the inorganic phosphorus varied within wide limits. Many of the blood samples were taken soon after the animals were placed on calcium-deficient rations, and therefore some of the samples, though only a small proportion, were about normal, whereas in certain extreme cases they were below 5 mg. The same wide variations are found in the inorganic phosphorus; however, except for pigs under 7 months old most of the results are above the average for normal pigs of the same age. The statements concerning wider variations than for normally fed pigs is borne out by the standard deviation from the mean.

When the older animals were low in serum calcium for a long time it was very difficult to get them pregnant, and they often failed to conceive. This condition may have been due to a deficiency of vitamin A or to the combination of an insufficient quantity of this vitamin and calcium in the diet. Of those that did farrow many had weak undersized pigs that failed to grow normally, apparently because of a lack of normal milk production by their mothers.

The records of several individuals fed calcium-deficient diets for long periods follow.

Two sows, born in September 1929 and bred in November 1930, were fed a basal diet consisting of barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent. Such a diet contains a relatively small amount of calcium but probably enough

phosphorus for normal growth, and physical well-being.   It is low in vitamin A.   Both sows farrowed in March 1931, and one farrowed again in October 1931.   The other was due to farrow in December of the same year but did not.   Neither farrowed thereafter, though fertile boars were put into the pens with them.   Serum calcium and inorganic phosphorus were first determined before the sows farrowed in March.   Since the records of these two sows are very similar, only one (for 7–F–29) is given in detail (table 9).

TABLE 9.—*Calcium and inorganic phosphorus in 100 cc of blood serum of sows 7–F–29, 6–F–29, and 32–F–30 while on the diets indicated*

SOW 7–F–29 [1]

Diet	Date of sampling	Calcium	Phosphorus
	1931		
		Milligrams	*Milligrams*
	Mar. 7	11.35	9.19
	Mar. 16	10.90	8.76
	Apr. 9	11.00	10.10
	June 3	7.55	11.19
	July 24	6.50	8.88
Basal, from Nov. 10, 1930, to Dec. 20, 1931	July 31	6.80	8.62
	Oct. 12	6.29	10.37
	Oct. 13	6.50	9.47
	Oct. 15	5.05	9.76
	Nov. 9	5.75	9.70
	Nov. 10	4.75	10.22
	Dec. 19	5.00	9.14
	Dec. 21	6.35	8.13
Basal, plus 1.5 percent of CaCO₃ from Dec. 20, 1931, to Jan. 5, 1932	Dec. 23	6.85	8.81
	Dec. 28	7.90	8.83
	1932		
	Jan. 4	9.18	8.96
Basal, from Jan. 5–14, 1932	Jan. 7	6.23	10.15
	Jan. 11	5.11	10.94
Basal, plus 1.5 percent of CaCO₃ from Jan. 14–27, 1932	Jan. 15	7.91	10.51
	Jan. 25	9.78	7.97
Basal, from Jan. 27 to Feb. 8, 1932 (no sunlight)	Feb. 8	6.94	10.30
	Feb. 15	7.05	10.52
Basal, plus 6 drops of viosterol daily from Feb. 8 to Mar. 29, 1932	Mar. 1	8.22	10.57
	Mar. 8	8.21	
	Mar. 25	8.52	
	Apr. 20	10.61	7.77
	May 15	7.25	8.42
Basal, from Mar. 29 to Dec. 22, 1932	June 14	6.06	9.20
	July 11	6.55	8.89
	Sept. 13	8.00	9.76
	Dec. 22	8.48	12.55

SOW 6–F–29

Diet	Date of sampling	Calcium	Phosphorus
	1931		
	Oct. 15	10.10	7.08
	Nov. 9	6.15	9.14
	Nov. 10	4.95	9.52
	Dec. 9	5.55	10.00
	Dec. 19	5.90	8.04
Basal, from June 14, 1931, to Feb. 18, 1932 (no sunlight)	1932		
	Jan. 11	5.56	9.49
	Feb. 8	7.46	9.14
	Feb. 15	5.72	7.87
	Feb. 18	7.25	[2] 11.62

[1] This sow farrowed a normal litter of 8 pigs Mar. 15, 1931, that weighed 22 pounds.   The pigs were weaned May 15, of the same year.
[2] The blood sample taken immediately after death.

TABLE 9.—*Calcium and inorganic phosphorus in 100 cc of blood serum of sows 7-F-29, 6-F-29, and 32-F-30 while on the diets indicated*—Continued

SOW 32-F-30 [3]

Diet	Date of sampling	Calcium	Phosphorus
	1931	*Milligrams*	*Milligrams*
Normal herd ration	July 31	11.50	7.06
	Dec. 19	11.45	7.15
	1932		
	Apr. 21	12.02	7.19
	May 9	7.19	8.91
	May 15	6.51	8.29
Basal (barley, 60 percent; wheat middlings, 25 percent; wheat bran,	May 23	6.55	8.00
14 percent; and salt, 1 percent) from Dec. 19, 1931, to Oct. 4, 1932	May 31	7.53	8.29
(no sunlight).	June 14	7.43	9.41
	July 11	6.45	8.12
	Oct. 4	5.30	9.47

[3] On Apr. 9, 1932, farrowed 10 normal pigs; late in May became very nervous; by June 14 all the pigs had died.

Not until these sows lactated did their blood calcium show a marked reduction below normal. One of them showed a lack of coordination of the hindquarters when the pigs were weaned and for a time was unable to get up alone, but she gradually regained her strength and after 5 days was able to walk again. The serum calcium of sow 7–F–29 became very low in the fall of 1931. The addition of 1.5 percent of $CaCO_3$ resulted in an immediate but slow rise in the serum calcium. A return to the basal diet caused a decrease in the calcium content of the serum at once. The giving of 6 drops of viosterol daily in the absence of sunlight caused a gradual rise in the serum calcium. A change back to the basal ration, however, resulted in a lowering of the serum calcium during the summer. By late December 1932 both sows were in such bad condition that they were killed. Neither was pregnant.

When the pigs from these sows were weaned in May 1931, they were fed the same basal ration as their mothers, and this diet was continued until August 5. At no time after weaning did the pigs appear normal. Their condition was poor, their hair was dry and curly, and they seemed to have an abnormal desire for water. After August 5, they were divided into two groups. One group was fed the basal diet and the other was fed the basal diet to which was added 1.5 percent of $CaCO_3$. Neither group did well, and by September 13 only one pig in each group was alive. The rest of the pigs had died either of pneumonia or what seemed to be necrotic enteritis. The serum calcium and inorganic phosphorus of these pigs were below normal for animals of their age. From September 13, 1931, to January 7, 1932, the two pigs still alive were fed the basal ration plus 1.5 percent of $CaCO_3$ and each was given 10 cc of cod-liver oil daily. They were finally turned out to pasture, fed a normal diet, and marketed.

The second litter of the other sow of the pair was farrowed October 8, 1931, and the pigs were weak at birth. This sow had little or no milk when the pigs were born; however, on December 12 five of

the seven pigs born were weaned. After weaning they were put on the same basal ration as their mother, and by February 1, 1932, all of them had died. The average serum calcium and inorganic phosphorus of these pigs were: On November 25, 1931, calcium, 7.53 mg and phosphorus, 6.32 mg; on December 12, 1931, calcium, 7.03 mg and phosphorus, 6.40 mg; and on February 10, 1932, calcium, 6.82 mg and phosphorus, 6.25 mg. These pigs, like the others, were far below normal in serum calcium and inorganic phosphorus. The milk of the mother was not tested for calcium or phosphorus, and it is therefore impossible to postulate that the calcium and phosphorus content of the mother's milk was not normal; however, the evidence indicates that such was the case.

The record of a third sow, 6–F–29, shown in table 9, is presented because some of her pigs apparently died of calcium tetany. She farrowed a normal litter in September 1930, and another litter in March 1931. On June 14, 1931, she was put on the same low calcium diet that had been given to sow 7–F–29, and kept away from the direct rays of the sun. On September 18, 1931, she farrowed eight pigs that appeared normal at birth. Like sow 7–F–29, her blood calcium was very low, particularly during the lactation period, and, generally speaking, as her serum calcium decreased her inorganic phosphorus increased. Of the pigs farrowed on September 18 only one was alive by December 21. The rest had died in convulsions. These pigs while apparently still in good condition would act in an abnormal manner, and finally would lie down, thrash about, their muscles would contract, their legs would extend straight out from their body, and death would ensue. Their muscles did not relax after death. The blood calcium of two of these pigs taken after death was, respectively, 4.65 and 7.40 mg in 100 cc of serum. The serum calcium of the latter pig taken 14 days previously was 5.95 mg. On December 8 the one pig that lived after weaning had a serum calcium and inorganic phosphorus content of 6.25 and 11.68 mg respectively. He failed to grow normally and in March 1932 was killed. On post-mortem examination it was found that one of his hip sockets was being resorbed.

The record of sow 32–F–30 is presented in table 9. On October 2, this pregnant sow seemed to be having nervous and muscular difficulties. A note taken from the original record reads: "She trembles all over, gasps for air, grunts loudly, lies down, jumps up suddenly, sits down with front legs stretched in front of her, and appears short of breath." On October 3 her udder developed rapidly, and on the morning of October 4, a blood sample was taken. By afternoon she was unable to rise. The next day she lay on her right side until about 11 a. m., when she began thrashing about and breathing hard. At 1 p. m. she was dead. On post-morten examination, conducted a few minutes after death, six fetuses were removed and blood samples were taken. The fetuses were outwardly normal and weighed altogether 17.5 pounds. The sow had small infection areas in one lung and a slight necrosis of the small intestine. It is unlikely that either caused death; apparently she died of acute calcium tetany. The average serum calcium and inorganic phosphorus for five of the fetuses were, respectively, 19.58 mg and 17.24 mg.

Since the blood of these unborn pigs was taken after the death of the sow, asphyxiation may have influenced the quantity of inorganic phosphorus present. Collip (5) found that the inorganic phosphorus of asphyxiated fetuses was higher than that of normal fetuses and that asphyxiation caused a rise of about 1 mg in the blood of the dog.

The serum calcium of the mother taken on October 4 was less than one-third that of the fetuses taken the following day. Her inorganic phosphorus was only about one-half that of the fetuses she was carrying.

The following record shows the normal serum calcium of a sow and her litter during lactation. Sow 14–S–31 farrowed a litter of nine normal pigs April 9, 1932. The average of three samples of blood taken May 9, May 23, and June 14, 1932, was for the sow, serum calcium, 11.35 mg and inorganic phosphorus, 7.03 mg; for the pigs, serum calcium, 12.19 mg and the inorganic phosphorus, 7.86 mg.

SUMMARY

The mean serum calcium and inorganic phosphorus of the blood of normal pigs of all ages was found to be 11.93 and 8.34 mg respectively.

The serum calcium and inorganic phosphorus of the young pig was higher than for the older pig or the adult hog.

The serum calcium of the blood of the sow immediately before and after farrowing was higher, and the inorganic phosphorus slightly lower, than the mean for nonpregnant sows of the same age.

The administration of calcium salts directly into the stomach of the pig caused a temporary rise in the serum calcium.

Continuous feeding of a relatively large proportion of calcium carbonate in the diet caused a prolonged rise in the serum calcium.

As the serum calcium increased above the normal there was at the same time usually a decrease in the inorganic phosphorus.

Continuous feeding of a ration with a low calcium content over a long period of time resulted in a marked lowering of serum calcium.

As the serum calcium decreased below the normal there was at the same time a fairly regular increase in the inorganic phosphorus in the serum of mature animals, but in young pigs there was a decrease.

Evidences of calcium tetany were manifested when the serum calcium became very low.

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NUTRITIVE VALUE OF THE PROTEIN OF CAJANUS INDICUS[1]

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INTRODUCTION

In Hawaii, the pigeonpea (*Cajanus indicus*) is a legume much prized for stock feeding but used only occasionally for human food. The agronomic aspects of this legume in Hawaii have been fully discussed by Krauss (8).[2] In some other tropical and semitropical countries, notably in India, the pigeonpea constitutes an important human food.

Since the work here reported was begun, two reports dealing with the biological value of the proteins of pigeonpeas have appeared in the literature. Sundaram, Norris, and Subrahmanyan (17) have prepared from the decorticated seeds two globulins, one, which they call cajanin, accounts for 58 percent of the total nitrogen of pigeon-peas, and the other, concajanin, accounts for 8 percent. They also found an albumin which constitutes about 4 percent of the total nitrogen. Their summary states that these three proteins contained requisite amounts of cystine; the quantities of cystine reported in their paper—presumably percentages on the ash- and moisture-free basis—are 2.73 for the albumin, 2.24 for concajanin, and 1.41 for cajanin, all determined by the method of Folin and Looney. Slightly different figures were obtained by the Sullivan method. These authors report no feeding experiments.

Another report published 2 years later by Niyogi, Narayana, and Desai (12) gives the results of analysis of the mixed globulins of pigeonpeas and biological values for the pure globulins and the whole seed when fed at a 5-percent protein level by the Mitchell method. In the summary of their article these authors state that the globulins have been found to be deficient in cystine and tryptophane. Their report contains a table giving the quantities of the important amino acids, which it definitely states are expressed as percent of protein—ash- and moisture-free. Cystine is given as 1.58 percent.

Considering the quantities of cajanin and concajanin, which the first-mentioned authors report, one may conclude that the mixed globulins prepared by the two groups contain very nearly the same quantity of cystine, but that the authors of the two reports differ in their interpretation as to what constitutes a "requisite" amount of cystine. However, the quantity of cystine reported in both papers seems very high for legume proteins. The cystine in a number of legume-seed globulins has recently been redetermined by the Bureau of Chemistry and Soils, United States Department of Agriculture, and in no case was it found to exceed 0.58 percent, the average for seven globulins being 0.35 percent.[3]

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Both reports agree that tryptophane is low in the important globulin, cajanin, and in the mixed globulins; the figure for the latter on the ash- and moisture-free basis being 0.46 percent.

It is usually considered that casein has the requisite amount of cystine, although Sherman and Woods have shown it to be the first "limiting" amino acid (*15*). The cystine content of casein is given variously as 0.20, 0.30, 0.26, and 0.66 percent according to the method used for the determination (*19*).

The work here reported deals with the nutritive value of a meal prepared from the whole (undecorticated) seed of pigeonpeas grown in Hawaii; the preparation and analyses of the mixed globulins prepared from this meal; the results of feeding experiments designed to illustrate the growth and reproduction of rats fed the seed meal as the sole source of protein; and the results of using the paired-feeding method of Mitchell (*11*) in which the diet of one animal is supplemented with one or more amino acids, in this case cystine, and for one small series of experiments, cystine and tryptophane.

A variety of pigeonpea grown on the University Farm and known as strain D was used throughout the tests.

PREPARATION AND ANALYSIS OF GLOBULINS

Preliminary trials showed that under the conditions and temperatures at which it was necessary to work, the greatest quantity of protein could be extracted by using the procedure described below.

Twelve liters of 5-percent sodium chloride solution were added to 2,400 g of finely ground seed meal and stirred continuously with a wooden paddle for one-half hour. The mixture was allowed to stand for $3\frac{1}{2}$ hours, after which it was strained through two thicknesses of cheesecloth and the remaining liquid pressed out in a small strong hand press until the residue was almost dry. No second extraction of the meal was made. The extract, which appeared muddy and opaque, was allowed to stand for an hour or longer and the liquid was decanted from the starch and other materials which settled to the bottom of the flasks.

By putting the decanted liquid through thick pads of paper pulp on large Büchner funnels 10 or more times, an almost water-clear, though somewhat opalescent, filtrate was obtained. Approximately $10\frac{1}{2}$ liters of the clear filtrate were obtained from each lot of 2,400 g of seed meal so extracted.

Considering the conditions for working and the quantities of material involved, dialysis of the filtrate was impractical and dilution and slight acidification were used. To each 1,000 cc of the filtrate, 4 cc of 10-percent acetic acid and 5 liters of distilled water were added, the mixture was stirred thoroughly, placed in large precipitating jars, and kept in a refrigerator overnight.

The following morning the water was siphoned off from the layer of precipitated protein and more distilled water was added. The second morning after siphoning off the water, several lots of protein were usually combined, 95-percent ethyl alcohol was added, and the mixture allowed to stand for 24 hours. The alcohol was removed by decanting and filtering, fresh 95-percent alcohol added, and the mixture again allowed to stand 24 hours. After four such successive washings with 95-percent alcohol, the protein was given one washing

with absolute alcohol which was removed after 24 hours and redis-
tilled ether added. After 24 hours the ether was removed by suc-
tion and the protein was dried in a desiccator over sulphuric acid.
Any attempt to shorten the process at any point resulted in an end
product that was hard and horny. With the process used, the protein
appeared as a fine, grayish-white, amorphous powder. The yield for
various lots extracted at different times varied from 3.5 to almost
4 percent of the weight of the meal used.

Analyses of a composite sample representing the mixed globulins of
the pigeonpea made up from the product of several sets of extractions
showed the protein to have 0.73 percent ash on the moisture-free basis.
The following tabulation shows the composition—ash- and moisture-
free—of the mixed globulins prepared as described above:

	Percent
Carbon	50.630
Hydrogen	6.930
Nitrogen	16.410
Sulphur	.450
Oxygen	25.580
Cystine [4]	.362
Tryptophane [4]	.367

[4] Analysis furnished by D. Breese Jones, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

Sulphur was determined by the official sodium peroxide method *(1)*,
cystine by the Sullivan method *(16)*, and tryptophane by the method
of May and Rose *(10)*.

These figures show protein prepared as above to contain about the
same quantity of carbon, hydrogen, nitrogen, sulphur, and trypto-
phane as the globulins of pigeonpeas previously reported, but much
less cystine.

FEEDING EXPERIMENTS

GROWTH RESPONSE OF RATS FED COOKED AND UNCOOKED CAJANUS SEED MEAL

In 1921 Waterman and Jones *(20)* pointed out that the digestibility
of legume proteins was improved by cooking, and in many later
experiments *(3, 4, 6, 7)* showed that rats fed cooked legumes fared
better than those fed raw legumes.

The finely ground *Cajanus* seed meal for these experiments was
cooked without addition of water by placing a 2-inch layer in a pan
on a rack in a pressure cooker and heating for 1 hour at 15 pounds
pressure. The product, which was slightly damp, was dried at room
temperature and reground.

A group of four rats, weaned at 28 days from mothers on a good
breeding diet, was fed for 10 weeks diet no. 1 of the following com-
position: Raw pigeonpea-seed meal, 90 percent; Osborne and Mendel
salt mixture, 3 percent; Crisco, 6 percent; and sodium chloride, 1 per-
cent. In addition, the rats were fed 10 drops (0.24 g) of cod-liver oil
and 8 drops of tikitiki (equivalent to 1 g of rice bran) daily except
Sunday. Four animals of the same litter were fed diet no. 2 of the
same composition except that the seed meal was cooked. In each
case the *Cajanus* meal supplied approximately 18 percent of protein
in the diet. The contrasting growth curves of the two groups are
shown in figure 1. The rats fed the cooked pigeonpea-seed meal
gained much more in weight and were in much better condition at
the end of the experiment than were the rats fed the raw meal.

GROWTH RESPONSE OF RATS FED COOKED CAJANUS MEAL AS THE SOLE SOURCE OF
PROTEIN COMPARED WITH GROWTH RESPONSE OF RATS FED BREEDING DIET

Figure 2 illustrates the growth response of additional animals fed
the cooked seed meal diet described above. Separate curves for males
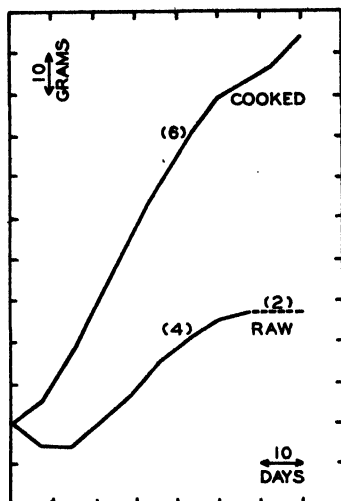
FIGURE 1.—Growth curves of rats fed raw
and cooked *Cajanus indicus* meal as the
sole source of protein (diets 1 and 2). Fig-
ures in parentheses refer to the number of
rats used for each test. The broken line
indicates the death of one or more rats.
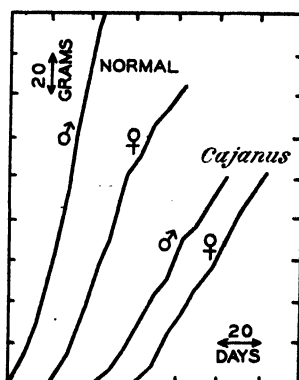
FIGURE 2.—Growth curves of typical
rats fed cooked *Cajanus indicus* seed
meal (diet 2) as the sole source of pro-
tein, compared with growth curves
of normal rats fed a good breeding
diet containing whole-milk powder.

and females are shown in comparison with growth curves of normal
(breeding) animals in the laboratory.
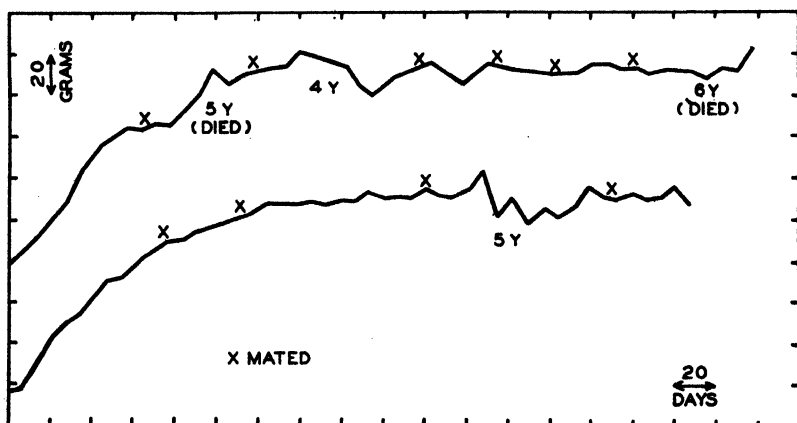
FIGURE 3.—Typical growth curves and breeding record of two female rats fed *Cajanus indicus* meal as the
sole source of protein (diet 2). Y means young born.

REPRODUCTION ON CAJANUS MEAL DIET

Four females and two males fed diet no. 2 were used for breeding
purposes. Figure 3 illustrates typical growth curves and the breed-
ing record of two of the females fed diet no. 2. Frequent matings
resulted in only an occasional pregnancy and the young often died.

GROWTH OF SECOND-GENERATION RATS ON CAJANUS MEAL DIET

Figure 4 illustrates the growth curves of second-generation animals on diet no. 2. They were given five drops of cod-liver oil and 0.2 g of dry yeast daily in addition to the cooked *Cajanus* meal diet. The growth response of these rats is definitely inferior to that of first-generation animals (fig. 2). They did not breed and no third-generation animals could be obtained on this diet.

COMPARISON OF CAJANUS MEAL AND CASEIN AS THE SOLE SOURCES OF PROTEIN

Young rats of the same litters, paired for weight and sex, were placed on the following diets at the age of 4 weeks: Diet no. 3—cooked *Cajanus* meal, 37 percent (furnished 8 percent of

FIGURE 4.—Growth of second-generation rats fed *Cajanus indicus* seed meal as the sole source of protein (diet 2). Figures in parentheses indicate the number of rats used for each test. Broken lines indicate the death of one or more rats.

protein in the diet); starch, 53 percent; Crisco, 6 percent; and Osborne and Mendel salt mixture, 4 percent. Diet no. 4—casein, 10.2 percent (furnished 8 percent of protein in the diet); cornstarch, 79.8 percent; Crisco, 6 percent; and salt mixture, 4 percent. In addition to the diets allowed ad libitum, 10 drops of cod-liver oil and 0.4 g of dry yeast were fed daily except Sunday. The results of feeding these diets for 9 weeks are summarized in figure 5. The average food consumption of the female rats fed diet no. 3 was about 4 percent greater, and that of the male rats about 10 percent greater, than the average food consumption of the corresponding groups of rats fed diet no. 4.

It took the rats fed *Cajanus* meal about a week to adjust themselves to the vegetable-protein diet, but after that they grew at a rate that compared favorably with that of the rats on animal protein.

FIGURE 5.—Comparative growth curves of rats fed *Cajanus indicus* seed meal or casein as source of protein (diets 3 and 4 with total protein content equaling 8 percent). Figures in parentheses indicate the number of rats used for each test.

ARE THE PIGEONPEA-SEED MEAL AND THE GLOBULIN PREPARED FROM IT DEFICIENT IN CYSTINE?

Paired-feeding experiments were made to determine the effect of adding cystine to a diet in which pigeonpea-seed meal furnished the sole protein. Mitchell and his co-workers have shown (*2, 11*) that most legumes are deficient in this amino acid. The composition of the diets is shown in table 1.

TABLE 1.—*Composition of rations*

Diet and percentage of protein	*Cajanus* seed meal [1]	Crisco	Salt mix-ture [2]	Corn-starch	Cystine	Sodium chloride	Cod-liver oil	*Cajanus* globulins	Butter
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Diet 5, 8 percent	37	6	4	53					
Diet 6, 8 percent	37	6	4	52.7	0.3				
Diet 7, 8 percent	37	6	4	52		1			
Diet 8, 8 percent	37	6	4	51.7	.3	1			
Diet 9, 11 percent	55	6	4	32		1	2		
Diet 10, 11 percent	55	6	4	31.7	.3	1	2		
Diet 11, 10 percent			4	74.4			2	11.6	8
Diet 12, 10 percent			4	74.1	.3		2	11.6	8

[1] Actual percentage of meal varied as moisture content varied. Each lot of cooked meal was analyzed for protein (N × 6.25) and moisture.
[2] Osborne and Mendel (*13*).

Summarized in table 2 are the results of feeding 35 pairs of rats for 10 weeks. Pairs numbered 1 to 11 received diets 5 and 6, and pairs numbered 12 to 35 received diets 7 and 8. These diets furnished 8 percent of protein from the pigeonpea-seed meal. In addition, the rats were fed 0.2 g of dried yeast daily except Sunday.

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*

8 PERCENT CAJANUS INDICUS PROTEIN

Pair	Food consumed	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
No. 1:					
Control	447	59	88	29	
Cystine	434	59	103	44	+15
No. 2:					
Control	354	59	85	26	
Cystine	291	52	58	6	−20
No. 3:					
Control	363	53	65	12	
Cystine	359	52	81	29	+17
No. 4:					
Control	478	70	110	40	
Cystine	456	71	105	34	−6
No. 5:					
Control	464	66	98	32	
Cystine	475	64	118	54	+22
No. 6:					
Control	426	58	94	36	
Cystine	418	58	102	44	+8
No. 7:					
Control	510	63	103	40	
Cystine	453	61	93	32	−8
No. 8:					
Control	459	60	74	14	
Cystine	428	58	91	33	+19
No. 9:					
Control	595	57	93	36	
Cystine	482	55	90	35	−1
No. 10:					
Control	558	52	84	32	
Cystine	488	48	87	39	+7
No. 11:					
Control	377	51	66	15	
Cystine	496	51	82	31	+16
No. 12:					
Control	540	60	96	36	
Cystine	540	63	103	40	+4
No. 13:					
Control	531	56	95	39	
Cystine	531	54	90	36	−3

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*—Continued

8 PERCENT CAJANUS INDICUS PROTEIN—Continued

Pair	Food consumed	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
No. 14:					
Control	534	56	81	25	
Cystine	534	59	91	32	+7
No. 15:					
Control	422	57	72	15	
Cystine	422	56	66	10	−5
No. 16:					
Control	544	56	91	35	
Cystine	544	56	102	46	+11
No. 17:					
Control	506	50	84	34	
Cystine	506	57	78	21	−13
No. 18:					
Control	536	50	83	33	
Cystine	536	51	83	32	−1
No. 19:					
Control	480	56	85	29	
Cystine	477	56	90	34	+5
No. 20:					
Control	458	50	82	32	
Cystine	459	50	94	44	+12
No. 21:					
Control	360	40	58	18	
Cystine	362	44	65	21	+3
No. 22:					
Control	381	49	65	16	
Cystine	379	50	65	15	−1
No. 23:					
Control	376	52	62	10	
Cystine	376	54	70	16	+6
No. 24:					
Control	354	50	60	10	
Cystine	354	49	56	7	−3
No. 25:					
Control	429	50	67	17	
Cystine	430	50	62	12	−5
No. 26:					
Control	401	44	58	14	
Cystine	400	44	68	24	+10
No. 27:					
Control	409	62	78	16	
Cystine	414	62	90	28	+12
No. 28:					
Control	384	59	75	16	
Cystine	379	60	75	15	−1
No. 29:					
Control	472	58	91	33	
Cystine	472	56	90	34	+1
No. 30:					
Control	472	58	80	22	
Cystine	472	60	91	31	+9
No. 31:					
Control	424	51	86	35	
Cystine	417	51	70	19	−16
No. 32:					
Control	461	50	74	24	
Cystine	460	50	74	24	0
No. 33:					
Control	446	58	86	28	
Cystine	450	56	98	42	+14
No. 34:					
Control	446	52	83	31	
Cystine	444	52	83	31	0
No. 35:					
Control	435	50	78	28	
Cystine	436	48	82	34	+6

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*—Continued

11 PERCENT CAJANUS INDICUS PROTEIN

Pair	Food consumed	Final body length	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*
No. 1:						
Control	532	17.8	68	129	61	
Cystine	531	17.3	66	134	68	+7
No. 2:						
Control	507	16.7	64	124	60	
Cystine	509	18.0	65	138	73	+13
No. 3:						
Control	471	16.4	62	108	46	
Cystine	474	17.5	62	137	75	+29
No. 4:						
Control	442	16.4	58	106	48	
Cystine	443	16.4	60	107	47	−1
No. 5:						
Control	459	16.3	58	103	45	
Cystine	459	17.0	59	118	59	+14
No. 6:						
Control	569	16.7	57	110	53	
Cystine	569	16.8	57	134	77	+24
No. 7:						
Control	435	15.4	51	98	47	
Cystine	438	16.3	54	110	56	+9
No. 8:						
Control	483	16.7	63	116	53	
Cystine	485	17.6	61	135	74	+21
No. 9:						
Control	450	15.8	55	108	53	
Cystine	450	16.0	55	112	57	+4
No. 10:						
Control	488	16.8	60	111	51	
Cystine	489	17.1	62	121	59	+8
No. 11:						
Control	462	16.5	53	99	46	
Cystine	462	15.4	48	97	49	+3
No. 12:						
Control	500	16.3	57	110	53	
Cystine	500	15.8	64	103	39	−14
No. 13:						
Control	474	15.5	54	94	40	
Cystine	474	15.9	50	104	54	+14
No. 14:						
Control	436	15.8	49	99	50	
Cystine	440	16.2	47	103	56	+6
No. 15:						
Control	458	16.4	51	108	57	
Cystine	448	16.1	48	102	54	−3
No. 16:						
Control	429	15.5	44	94	50	
Cystine	429	15.8	47	100	53	+3
No. 17:						
Control	407	14.8	42	94	52	
Cystine	471	16.3	42	100	58	+6
No. 18:						
Control	365	16.4	69	105	36	
Cystine	365	17.1	69	115	46	+10
No. 19:						
Control	353	15.9	67	103	36	
Cystine	353	17.1	66	107	41	+5
No. 20:						
Control	325	15.8	58	95	37	
Cystine	325	15.3	56	96	40	+3
No. 21:						
Control	337	15.8	62	86	24	
Cystine	337	16.2	61	107	46	+22
No. 22:						
Control	343	16.0	61	96	35	
Cystine	343	15.8	61	103	42	+7
No. 23:						
Control	388	16.8	66	110	44	
Cystine	388	16.7	66	129	63	+19
No. 24:						
Control	392	16.2	59	131	72	
Cystine	392	16.7	61	127	66	−6
No. 25:						
Control	316	15.4	59	86	27	
Cystine	316	15.6	58	97	39	+12

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*—Continued

11 PERCENT CAJANUS IDICUS PROTEIN—Continued

Pair	Food consumed	Final body length	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
No. 26:	*Grams*	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*
Control	319	15.6	56	87	31	
Cystine	319	15.1	55	96	41	−19
No. 27:						
Control	388	17.6	65	137	72	
Cystine	388	17.2	65	133	68	−4
No. 28:						
Control	422	17.1	60	131	71	
Cystine	422	16.7	59	126	67	−4
No. 29:						
Control	461	17.5	54	138	84	
Cystine	461	16.6	54	120	66	−18
No. 30:						
Control	380	16.4	52	111	59	
Cystine	380	16.2	52	114	62	−3
No. 31:						
Control	379	16.5	63	125	62	
Cystine	379	16.5	62	122	60	−2
No. 32:						
Control	469	17.1	60	124	64	
Cystine	469	18.2	61	151	90	+26
No. 33:						
Control	406	16.5	55	106	51	
Cystine	406	16.7	56	125	69	−18
No. 34:						
Control	380	16.2	52	109	57	
Cystine	380	15.7	52	107	55	−2

The results of these experiments would indicate that cystine has only a slight supplementary value. A statistical analysis of the results by Love's modification of Student's method (9) shows that the odds are somewhat greater than 25:1 in favor of cystine. The food consumption of the first 11 pairs is not so uniform as it should be, the total intake varying considerably in several cases. If these 11 pairs are eliminated and the statistical analysis made on the remaining 24 pairs, the odds are only 11:1 in favor of cystine.

It was thought that possibly tryptophane or some other amino acid was the limiting factor when the seed meal was fed at a level to provide 8 percent of protein. Additional experiments were therefore planned in which the seed meal furnished protein equal to 11 percent of the diet. The results of feeding diets 9 and 10 to 34 pairs of rats are summarized in table 2. Pairs numbered 1 to 17 were fed for 8 weeks and pairs 17 to 34 for 6 weeks. Statistical analyses of these results show cystine to have a definite supplementary value, as the odds are greater than 1,110:1 in favor of cystine.

When the prepared pigeonpea-seed meal protein representing the mixed globulins was fed for 8 weeks to 7 pairs of rats at a level of 10 percent (diets 11 and 12), the results were not significant for cystine, as the odds were only 2:1. The results are summarized in table 3. With the same diets (11 and 12), a second set of paired feeding experiments was carried out. At the end of a 5-week feeding period the results were not conclusive, as the odds were only 4.5:1 in favor of cystine. However, when 0.2 percent of tryptophane was added to the diet containing cystine (diet 12) and the same rats were fed for 2 weeks longer the results were very different. A statistical analysis of the results by Love's method showed the odds to be 216:1

in favor of the diet containing the two amino acids. The results, summarized in table 3, indicate very definitely that tryptophane is a limiting growth factor when the pure globulins are fed, but they do not indicate whether cystine is a limiting growth factor. Possibly the rats would have grown as well with tryptophane but without cystine as with both amino acids. Time and materials were not available for additional tests.

TABLE 3.—*Supplementing effect of cystine and of cystine and tryptophane on rations containing 10 percent of Cajanus indicus protein in the form of the mixed globulins*

CYSTINE

Pair	Food consumed	Final body length	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*
No. 1:						
Control	317	15.5	54	82	28	
Cystine	317	15.8	46	86	40	+12
No. 2:						
Control	290	14.5	46	72	26	
Cystine	284	14.7	46	77	31	+5
No. 3:						
Control	317	14.8	44	78	34	
Cystine	317	14.6	44	76	32	−2
No. 4:						
Control	313	15.3	56	84	28	
Cystine	319	15.3	56	80	24	−4
No. 5:						
Control	322	15.8	55	85	30	
Cystine	321	15.6	48	82	34	+4
No. 6:						
Control	309	15.2	53	80	27	
Cystine	307	14.8	53	78	25	−2
No. 7:						
Control	318	14.4	44	68	24	
Cystine	320	14.6	54	72	18	−6

CYSTINE, 5 WEEKS

Pair	Food consumed	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
No. 8:					
Control	215	55	72	17	
Cystine	215	55	69	14	−3
No. 9:					
Control	209	54	70	16	
Cystine	209	54	74	20	+4
No. 10:					
Control	193	55	70	15	
Cystine	193	55	70	15	0
No. 11:					
Control	199	53	68	15	
Cystine	199	53	75	22	+7
No. 12:					
Control	190	50	64	14	
Cystine	190	50	66	16	+2
No. 13:					
Control	158	48	54	6	
Cystine	158	48	53	5	−1

TABLE 3.—*Supplementing effect of cystine and of cystine and tryptophane on rations containing 10 percent of Cajanus indicus protein in the form of the mixed globulins*—Continued

CYSTINE AND TRYPTOPHANE, 2 WEEKS LONGER

Pair	Food consumed	Final body length	Final weight [1]	Total gain	Difference in gain between control and cystine plus tryptophane
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*
No. 8:					
Control	87	15.3	78	6	
Cystine and tryptophane	87	15.7	84	15	+9
No. 9:					
Control	85	15.0	76	6	
Cystine and tryptophane	85	15.2	82	8	+2
No. 10:					
Control	85	15.2	79	9	
Cystine and tryptophane	85	16.1	88	18	+9
No. 11:					
Control	69	15.0	72	4	
Cystine and tryptophane	69	15.6	86	11	+7
No. 12:					
Control	85	14.4	70	6	
Cystine and tryptophane	85	15.3	87	21	+15
No. 13:					
Control	72	13.8	63	9	
Cystine and tryptophane	72	13.8	68	13	+4

[1] Initial weight is final weight in the table above.

DISCUSSION

The tabulation on page 283 shows that the sulphur content of the mixed globulins is 0.45 percent and the cystine content 0.362 percent. The cystine accounts for only 19 percent of the sulphur. In what form does the remaining sulphur exist? If it is in the form of methionine, it is probable that it can be utilized as effectively as though it were in the form of cystine, as Jackson and Block (5) have shown that methionine greatly stimulated the growth of rats subsisting on a cystine-poor ration.

An arresting comment on the growth response of rats fed definite amounts of cystine has recently been made by Swift, Kahlenberg, Voris, and Forbes (18). They state that when their rats received a total of 0.022 g of cystine per week in their rations, the addition of more cystine did not result in increased growth.

Careful checking of the feeding experiments reported in this paper indicates that all the rats fed the diets without cystine supplements received a rather high intake of sulphur and that, even though not all of it is in the form of cystine or methionine, the intake of these two substances must have been much in excess of 0.022 g per week. For example, in the experiments in which seed meal was fed to provide 11 percent of protein, only about 8 percent of the sulphur would need to be in the form of cystine in order to furnish 0.022 g per week. As the writers have in every case fed 0.2 g of dried yeast daily except Sunday, 0.00324 g of cystine per week would have been added from this source, using the value of 0.270 percent cystine for dry yeast which the above-mentioned authors (18) report.

In the experiments reported by Mitchell and Smuts (11), if only one-fourth of the sulphur in soybeans were in the form of cystine, the rats would have received a little more than 0.024 g of cystine per week, yet they concluded from their feeding experiments that soybeans are deficient in cystine. The soybean meal used by them was

ether-extracted but not cooked and the rats ate small quantities of the ration. One wonders what the results might have been with cooked soybeans and better food intake. Piper and Morse (*14*) report soybeans to contain 0.444 percent of sulphur. The station pigeonpea-seed meal contained 0.22 percent of sulphur.

SUMMARY

Although first-generation rats made good growth, second-generation rats made only fair growth when pigeonpea-seed meal was fed at a level calculated to provide 18 percent of protein in the diet. Reproduction was obtained with first-generation rats fed cooked *Cajanus* meal, although the record was unsatisfactory. No reproduction was obtained on second-generation rats fed the same diet. Pigeonpea protein is therefore not adequate for normal growth and reproduction of rats.

When pigeonpea-seed meal was fed at a level calculated to furnish 8 percent of protein, the addition of cystine did not materially improve the growth of rats, but when the seed meal furnished 11 percent of protein the addition of cystine markedly improved the growth of rats.

The first limiting growth factor of the prepared pigeonpea globulins appears to be tryptophane.

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STUDIES ON THE INHERITANCE OF RESISTANCE TO WILT (FUSARIUM NIVEUM) IN WATERMELON [1]

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INTRODUCTION

Wilt in watermelons (*Citrullus vulgaris* Schrad.) caused by *Fusarium niveum* (E. F. Smith), takes a heavy toll each year from melon growers in many sections of the United States. Since the causal organism lives over in the soil for as long as 15 to 20 years, the most promising method of controlling the disease is breeding for resistance. However, the existence of many physiological forms of the causal organism, differing in their action on the host (8),[3] may necessitate the development of a resistant melon for each locality in which different forms are found. The history of the Conqueror watermelon (5) demonstrates that a variety may be resistant in one locality and susceptible in another.

The object of the investigations reported in this paper was to determine if possible how resistance to *Fusarium niveum* is inherited. Similar studies have been made of resistance to other plant diseases, but up to the present very little has been reported on watermelon wilt.

REVIEW OF LITERATURE

Orton (5) crossed the citron, which is resistant to *Fusarium niveum*, with the susceptible Eden variety of watermelon and obtained in succeeding generations a resistant melon of good quality, which was called Conqueror. This melon proved resistant in eastern Iowa but was susceptible in Oregon.

Porter (6) used citron, African forage, and Chinese melons in crosses with susceptible watermelons. The Chinese melon was resistant, the citron showed 18 percent of wilted plants, and the African forage melon was immune. The F_1 from these crosses was as susceptible as the susceptible parent. Resistance in the F_2 varied, depending on the degree of resistance of the resistant parent. Flesh quality of the resistant parent was dominant in F_1. Porter and Melhus (7) in 1929 stated that "the F_3 generation of Kleckley-citron (variety Kafir) crosses proved 86 percent resistant." The F_3 of a Kleckley Sweet × Conqueror cross showed considerable resistance.

EXPERIMENTAL MATERIAL

The parental material consisted of commercial Early Fordhook obtained from a seed firm, and a watermelon from Russia obtained by E. C. Sherwood through the Office of Plant Introduction of the

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United States Department of Agriculture. This Russian melon was the one used by Sleeth (*8*) in his studies. It is inedible and has a white flesh which is very tough and bitter to the taste. As compared with the Early Forkhook, the Russian vines grow more vigorously and have longer internodes and coarser leaves. The flowers appear considerably later and the fruits are much longer than those of the Early Fordhook, which are nearly round. The seeds of the Russian are green, while those of Early Fordhook are nearly white. The Russian is resistant to wilt, while the Early Fordhook is susceptible. The Sweetheart variety, obtained from a seedsman, has been used as a check throughout the tests because of its extreme susceptibility.

Three physiologic forms of *Fusarium niveum* (nos. 5, 8, and 20) obtained from Sleeth (*8*), in pure culture were used in the greenhouse tests for the genetic analysis. Sleeth had found that form 5 from

FIGURE 1.—Photomicrograph of diakinesis in a microsporocyte of the Russian watermelon showing 11 pairs of chromosomes. × 970.

Oregon was least virulent, form 20 from North Carolina most virulent, and form 8 from Iowa was intermediate. These particular forms were chosen because they differed in virulence, were distinguishable morphologically, and had proved rather stable in Sleeth's work.

Kozhukhow (*3*) reported 11 pairs of chromosomes for several varieties of commercial watermelons. In the present study an examination was made of microsporoctye material from the Russian and Early Fordhook parents by the iron-acetocarmine smear method (*1*). The chromosome number in both cases was found to be 11 pairs. This correspondence in chromosome number was not unexpected since there was no sterility in the hybrids between the Russian and the commercial variety. At the pachytene stages th chromosomes did not stain very well by the method used, but the best preparations showed the chromosomes to be long, very thin, and considerably twisted. One of them appears to be associated with the nucleolus. At diakinesis the chromosomes are short, as may be seen in figure 1.

METHODS

In 1930 crosses were made at the West Virginia station between the Russian and Early Fordhook for the purpose of developing a commercial watermelon for West Virginia resistant to wilt. In 1932 the writer, with the material at hand, began a study of the mode of inheritance of resistance.

The F_1 was grown in 1931 when selfings and backcrosses to the Russian and Early Fordhook were made. In 1932 the F_2 and certain of the backcross progeny were grown in the field in soil that was artificially inoculated by covering the seeds with a mixture of the soil which Sleeth (8) had used in his greenhouse wilt tests. Several forms of the organism coming from various parts of the United States were present. The watermelon plants grown in this soil showed very little wilting. In addition, another planting was made · of the Early Fordhook and Russian backcrosses in uninoculated soil. Numerous selfings in both plots were made to produce F_3 and backcross families. There was a possibility that some susceptible genotypes were eliminated in the infested field. This source of error is felt to be of minor importance, however, since very little wilt appeared. Inasmuch as the parent varieties differed in several respects, detailed notes were taken at harvest on shape, flesh color and texture, and seed-coat color.

The genetic analysis of wilt resistance is based on the reaction (in infested soil in the greenhouse) of F_3 families, i. e., the progeny of selfed F_2 plants, and of backcross families, i. e., the progeny of selfed backcross plants. The testing of this material was started in the winter of 1932–33 and continued until the following winter.

Plantings were made in boxes 10 feet by 3 feet by 7 inches, filled with a rich loam soil which had been sterilized for about 4 hours under a large steam plan. After the second planting, sterilized sand was added to the soil to improve its texture.

Inoculum was prepared by growing the forms of *Fusarium* on rice for several weeks. It was then mixed with a bucket of sterile sand and thoroughly incorporated with the surface 3 inches of soil in the boxes. The plan was to run duplicate tests of each family with each of the three forms. Form 5 occupied boxes 1 and 2; form 8, boxes 3 and 4; and form 20, boxes 5 and 6. The boxes were separated from each other by at least 2 feet, and extreme care was exercised to prevent transfer of the infested soil. After working in soil inoculated with a certain form, the writer sterilized his hands for 2 or 3 minutes in 1:1,000 mercuric chloride solution and rinsed them in tap water before working in soil inoculated with a different form.

Before planting, the seeds were sterilized for one-half hour in a 1:1,000 solution of mercuric chloride. Twenty-five seeds were planted about 1½ inches apart in rows 4 inches apart. Each family was represented in each of the six boxes, thus comprising duplicate tests with each of the three forms.

The seed coat of the Russian seeds is much harder and thicker than that of the Early Fordhook, which makes the Russian slower in germinating. Segregation of this character would be expected and would cause some variation in the time of germination among the families.

As soon as wilt began to appear, seedlings showing the wilt symptoms were pulled at 2-day intervals and the number recorded. Throughout the experiment care was exercised to pull the seedlings at the same stage of wilting. Frequent isolations were made from the wilted plants to see whether they contained the form used in the inoculation. The seedlings were sterilized in a 1:1,000 solution of Hg Cl$_2$ for 5 minutes and then plated on Leonian's (4) fusarium agar. In all cases the original form was recovered from the wilted plants.

Thermographic records showed that the air temperature near the boxes fluctuated between 15° and 27° C. in winter and between 15° and 32° in summer. There was an occasional drop to 10° in winter and an occasional rise to 38° in summer. Sleeth (8) found that the optimum temperature for the growth of *Fusarium niveum* in culture was between 24° and 28°.

EXPERIMENTAL DATA

In the greenhouse tests of the parents and F$_1$, the Russian wilted 5.3, 26.1, and 16.7 percent, respectively, to forms 5, 8, and 20. The corresponding percentages for Early Fordhook were 4.5, 95.7, an 95.8; and for the F$_1$, 14.7, 83,3, and 68.5.
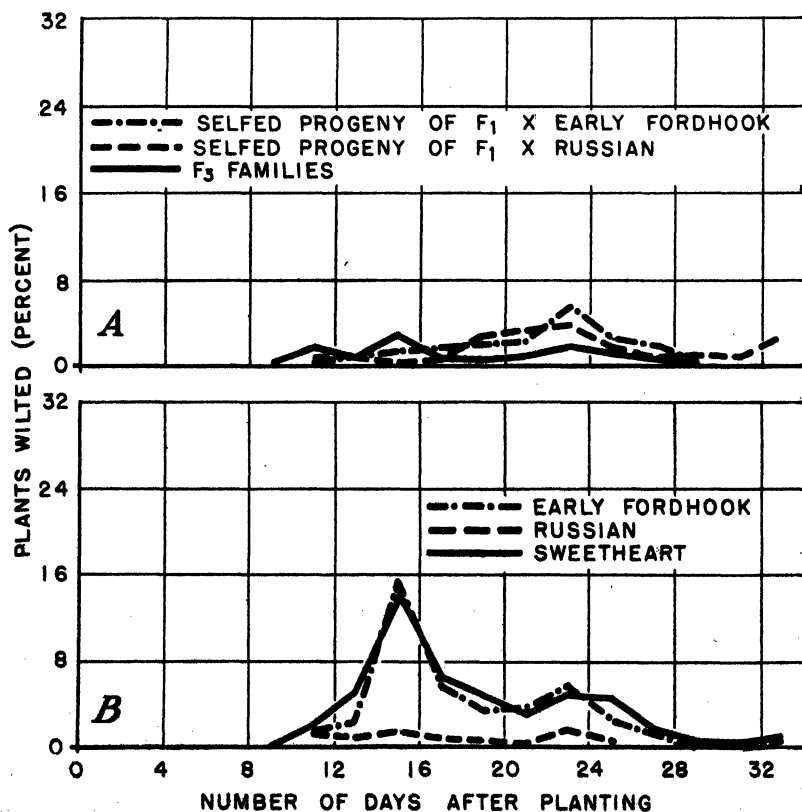
Figure 2.—Time and extent of wilt of watermelons when subjected in the greenhouse to form 5 of *Fusarium niveum: A*, Families descended from the Russian-Early Fordhook cross; *B*, parents and check.

INCIDENCE OF WILTED PLANTS AMONG PARENTS AND PROGENY

Since all the families could not be tested at the same time, in order to study the rate of wilting it was necessary to have some basis for comparing the results of different tests. Two methods were used. (1) Comparisons were made of the amount of wilt occurring on any given number of days after the first wilt had appeared in those particular tests. The Sweetheart check was used to determine this date of first wilting since it was the most susceptible variety. (2) Comparisons were made of the amount of wilt on any given number of
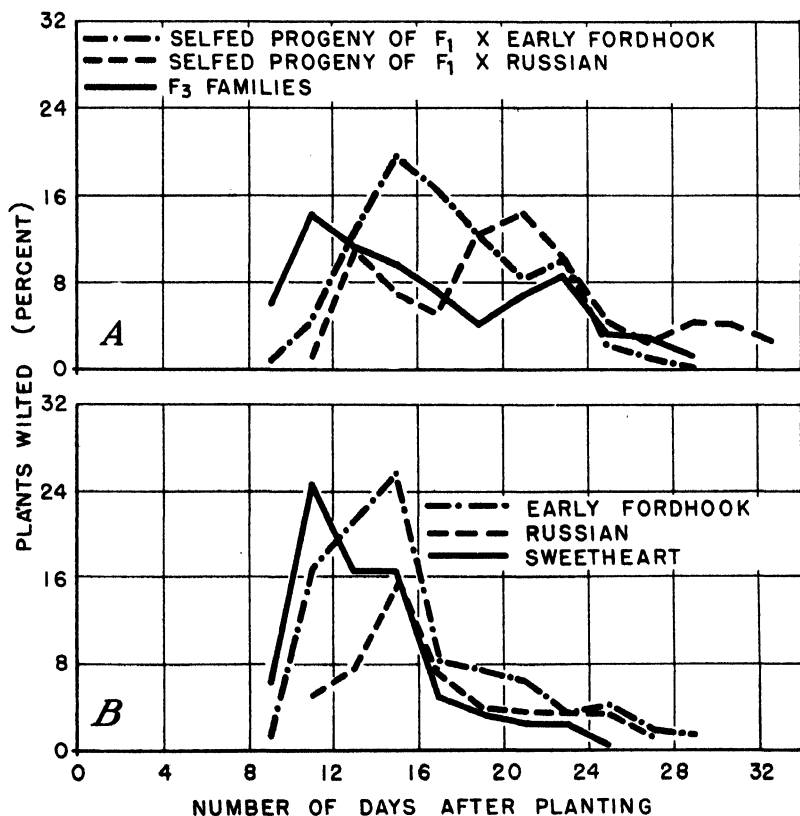
FIGURE 3.—Time and extent of wilt of watermelons when subjected in the greenhouse to form 8 of *Fusarium niveum: A*, Families descended from the Russian-Early Fordhook cross; *B*, parents and check.

days after planting. From one test to another there was frequently a variation of several days in the time from planting to the date of first wilting, and the first method was used to eliminate some of the effects of this variation.

The curves showing the incidence of wilt (based on time elapsed after planting) among the parents and Sweetheart (figs. 2, *B;* 3, *B;* and 4, *B*) indicate that in Early Fordhook the wilt proceeded more rapidly than in the Russian. The wilt in the Sweetheart check proceeded even more rapidly than that in Early Fordhook. In all cases there was more wilt in the Early Fordhook and Sweetheart

than in the Russian. This is noticeable particularly in the test with form 5, where there was very little wilt in the Russian (fig. 2, *B*)

Since the wilt (due to forms 8 and 20) that took place in the Russian parent occurred later than that in Early Fordhook, one would expect the families from backcrosses [4] to the Russian to show a delay in time of wilting. The curves (figs. 2, *A;* 3, *A;* and 4, *A*) for the back-cross and F_3 families show that this usually is the case. In the families from backcrosses to Early Fordhook the wilt occurred earlier than those in from backcrosses to the Russian. There is a suggestion
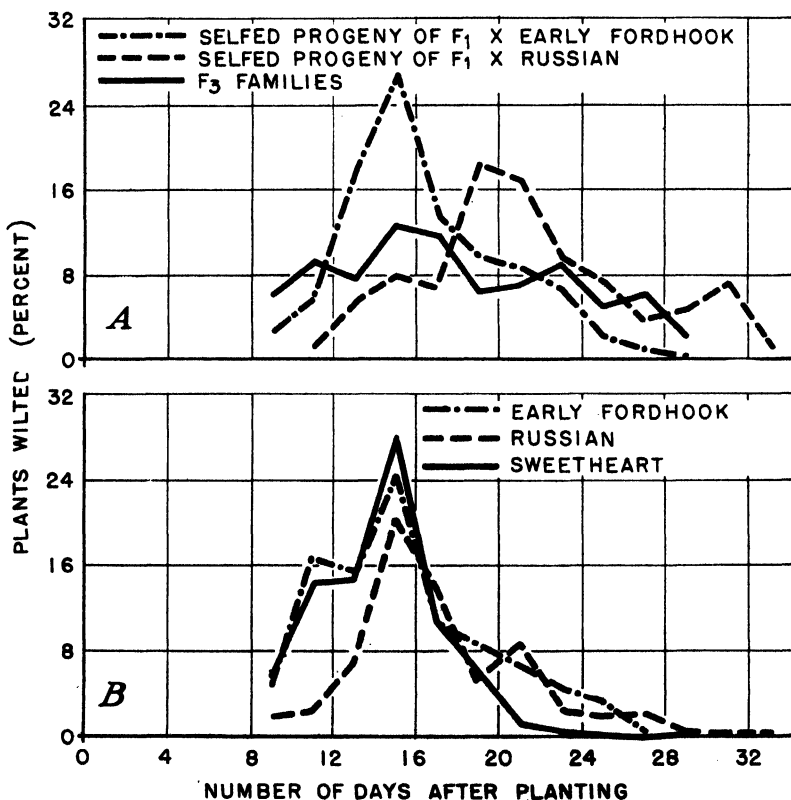
FIGURE 4.—Time and extent of wilt of watermelons when subjected in the greenhouse to form 20 of *Fusarium niveum: A,* Families descended from the Russian-Early Fordhook cross; *B,* parents and check.

of bimodalism in the reaction to form 8 (fig. 3, *A*) of the F_3 and the backcrosses to the Russian. This may indicate genetically distinct groups for resistance or a change in susceptibility at different stages of growth. As in the F_1 tests, form 5 caused very little wilt.

Four days after wilting began it was found that the peak of wilting in Early Fordhook had passed, whereas in the Russian it has not yet come. Consequently it was decided that the data on wilting obtained at the end of this 4-day period, in addition to that obtained at the end of the test, would be good material for use in analyzing the genetic difference between the Russian and Early Fordhook parents with regard to resistance.

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[4] Throughout this paper the term "backcross" is used to indicate the selfed progeny of the F_1 X parent unless otherwise stated.

DISTRIBUTION OF FAMILIES WITH RESPECT TO PERCENTAGE OF WILTED PLANTS
PER FAMILY

One hundred and eighteen families from the $F_1 \times$ Early Fordhook, 62 families from the $F_1 \times$ Russian, and 102 F_3 families were tested for resistance to each of the 3 fusarium forms. The results of these tests are shown in figures 5 and 6.

The tests of the F_3 and backcrosses to each parent were run at different times, which made it necessary to show the reaction of the parents during each test for purposes of comparison. The percentage of wilt is indicated by squares for the Russian parent, by circles for the Early Fordhook parent, and by triangles for the Sweetheart check. The position of the symbols directly above the bars indicates the average reactions of the parents and Sweetheart during the tests of the families which these bars represent. For example, the location of the square above the black bar in the 20–39.9 percent class (fig. 5, A) shows that the average percentage of wilted plants in the Russian parent during the tests of these Early Fordhook backcross families to form 20 fell in this class. Likewise it may be seen from this same distribution that the average percentages of wilted plants among the Early Fordhook parent and the Sweetheart variety fell, respectively, in the 40–59.9- and the 60–79.9-percent classes.

The distribution of the F_3 and backcross families with regard to the amount of wilt occurring during the first 4 days of wilting

FIGURE 5.—Histograms showing the distribution of families with respect to the percentage of wilted plant per family during the first 4 days after wilting appeared in the tests with the three forms of *Fusarium niveum*: A, form 20; B, form 8; C, form 5.

is shown in figure 5. It will be seen that with form 5 practically all the families in the F_3 and backcrosses to the Russian and Early Fordhook are in the lowest wilt class (0–19.9 percent), with some in the 20–39.9-percent class. Only a few families of the Fordhook backcross appear in the next class (40–59.9 percent). In other words, most of the families were relatively resistant to form 5, and there was no great difference between the reaction of F_3 or backcrosses to either parent. When tested with form 5 both parents and Sweetheart were in the lowest wilt class except in one case. This one exception was during the testing of the Russian backcrosses, when Fordhook and Sweetheart fell in the 40–59.9-percent class.

Form 8 was much more virulent, and when subjected to it more of the families showed a higher percentage of wilt. In fact nearly 10 percent of them fell in the 80–100-percent class. There was a larger percentage of the Russian than of the Early Fordhook backcrosses in the 0–19.9-percent class. This situation was reversed in the 20–39.9-percent class, while in the 40–59.9-percent class the Russian backcrosses again were in excess. In the two classes between 60 and 100 percent there was very little difference. The F_3 had the greater portion of its families in the low wilt class. The number in the progressively higher wilt classes gradually decreased without any distinct break. As with form 5, generally speaking, there was no great difference in reaction between the backcrosses to the two parents.

FIGURE 6.—Histograms showing the distribution of the families with respect to the total percentage of wilted plants per family during the tests with three forms of *Fusarium niveum*: *A*, form 20; *B*, form 8; *C*, form 5.

With form 20 the larger portion of the Russian backcross families was in the low wilt classes, with none in the 100-percent class, while the Fordhook backcross families were fairly well represented in all classes. There was a considerably smaller proportion of the Fordhook backcrosses in the low wilt classes than there was of the Russian backcrosses, showing very definitely the influence of the resistance of the backcross parent. The F_3 had a little over 30 percent of its families in the 0–19.9-percent wilt class, increasing to about 36 percent in the 20–39.9-percent class, then decreasing to 5 percent in the 80–100-percent class.

With forms 8 and 20 the Russian fell in lower wilt classes than did the Early Fordhook parent.

In order to determine the distribution and amount of wilt throughout the entire period, the tests were continued until most of the plants had wilted, and the number of plants remaining at the end of the test was recorded. In figure 6 is shown the proportion of families which fell in the various wilt classes based on the amount of wilt during the entire test. With form 5 the results are difficult to explain. The F_3 and Early Fordhook backcrosses had a larger proportion of families in the low wilt classes than did the Russian backcrosses, even though the Russian parent in all three cases showed a smaller amount of wilt than the Early Fordhook parent.

With forms 8 and 20 a larger proportion of the Russian than of the Fordhook backcross families were in the lower wilt classes, but an even greater proportion of the F_3 were in these classes.

With form 8 the Russian parent was in the 60–79.9-percent class, while the Early Fordhook was in the 80–100-percent class. With form 20 both parents were in the 80–100-percent class.

EVIDENCE OF TRANSGRESSIVE SEGREGATION

There were several families that showed greater resistance than the Russian parent and several that showed greater susceptibility than the Early Fordhook parent, indicating transgressive segregation.

CORRELATIONS

The correlations between the reactions to the three Fusarium forms were not high, as will be seen in table 1, but were generally higher at the end of the 4-day period than at the end of the test. There was a higher correlation between the reaction to forms 8 and 20 than there was between 5 and either 8 or 20. At the end of 4 days the correlation between the reaction to form 8 and the reaction to form 20 of the Russian backcross families is 0.72, while that between 5 and 20 is 0.48. According to Fisher's method (*2*) for testing the significance of differences between correlations, the difference in the z value for the two correlations is 0.45 ± 0.18. This is more than twice its standard error, indicating that the difference is significant. The correlation between the reaction to forms 8 and 20 of the F_3 families is greater than that between 5 and either 8 or 20 at the end of the 4-day period. Fisher's test shows that these differences are not significant.

INHERITANCE OF CERTAIN CHARACTERS

Notes were taken on certain characteristics of the plants as they were growing in the field and on the fruits at the time the seeds were removed. In the F_1 of the Russian × Early Fordhook and reciprocal crosses all of the Russian characters except fruit shape appeared to be dominant. The fruits borne on the F_1 plants were oval, as were those of the Early Fordhook parent. There appeared to be no simple segregation of the characters of the plants, although certain of the fruit characters suggested more definite ratios.

TABLE 1.—*Correlations between the action of the three Fusarium forms on the different groups of families* [1]

When correlated	Forms	Backcross to Early Fordhook (118 families)	F_3 (102 families)	Backcross to Russian (62 families)
At end of 4 days	5 and 8	0.14	0.54	0.46
	5 and 20	.15	.52	.48
	8 and 20	.32	.68	.72
At end of test	5 and 8	.21	.41	.02
	5 and 20	.04	.46	.09
	8 and 20	.53	.39	.45

[1] According to Fisher's V. A. table an r value of at least 0.1946 is necessary where $n = 100$ for odds of 19 to 1 ($P = 0.05$) for significance. For the same odds an r value of 0.2500 is necessary where $n = 60$.

There was considerable variation in the color of the flesh, and those fruits classified as red ranged from a slight pink to a bright red. The results from the backcrosses to Early Fordhook suggest a single factor

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THE TOXICITY OF CERTAIN CHEMICAL AGENTS TO ERWINIA AMYLOVORA [1]

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INTRODUCTION

Extensive toxicological studies have been made with fungi, both pathogenic and nonpathogenic, and much progress has been made in developing fungicides that are highly efficient in combating many plant diseases. However, much less attention has been given to similar studies with bacterial pathogens of plants. Considerable progress has been made in seed-stock treatments, soil treatments, and local disinfection of woody plant parts, tools, etc., to combat bacterial plant diseases; and limited advances have been made in spraying and dusting against these maladies, chiefly with materials developed as fungicides. Nevertheless, whether because of the difficulties inherent in the problem or because of the need for further investigation, plant pathologists still lack spraying or dusting methods for bacterial diseases that compare in efficiency with those now in common use against fungus diseases of plants.

Brooks (4) [2], Miller (17), Shaw (24), and Keitt, Pinckard, and Riker, as reported by Christensen (6, *pp. 86–88*), have made a series of studies bearing on the epidemiology and control of fire blight of apple (*Malus sylvestris* Mill.), caused by *Erwinia amylovora* (Burr.) Bergey et al., in Wisconsin. Under the conditions thus far encountered in this State, water-borne bacteria from nearby lesions have seemed to comprise a major inoculum for both primary and secondary natural infection. Furthermore, it has been shown (17) that, under favorable conditions, the bacteria can infect young leaves, blossom buds, or blossoms by way of the stomata, without the intervention of wounds. While it is realized that major factors in the epidemiology of fire blight may vary greatly with regional and seasonal conditions, the type of epidemiological evidence encountered in Wisconsin suggested that increased attention be given to the development of bactericidal spray or dust treatments to aid in the control of this disease. Accordingly, in 1929, work was initiated along two lines: (1) Laboratory studies of the toxicity of various chemical compounds and spray materials to the fire blight organism, and (2) orchard spraying experiments. Progress reports have been made on the earlier results (6, 11). An account of the laboratory work on toxicity follows.

The work here reported was undertaken with two major aims: (1) To extend to a bacterial plant pathogen the type of toxicological study that has been applied to bacterial pathogens of man or animals, and (2) to study the toxicity to *Erwinia amylovora* of various types of chemical compounds and spray preparations in the hope of gaining

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RESULTS FROM SPRAY MATERIALS

In addition to the work with molar solutions, tests were made of certain spray materials. The same technique was employed, except that dilutions were based on spray formulae, or, in the case of lime-sulphur, dilution by volume with water. The results will not be given in detail. The following excerpts are from the data from tests with the 10-minute medication period:

Bordeaux mixture.—In 15 experiments the formula of 0.5 pound $CuSO_4.5H_2O$, 1.5 pounds CaO, and water to make 50 gallons was always lethal; one-half of this concentration was never lethal.

Lime-sulphur (commercial liquid concentrate, 32° B.).—In six experiments at full strength it was always lethal. When diluted one-half with water, it was lethal in 50 percent of the tests. In no case was it lethal at 1 gallon in 20.

Zinc sulphate-lime spray.—In four experiments the formula of 2 pounds $ZnSO_4.7H_2O$, 1.5 pounds CaO, 0.75 pound of a commercial calcium caseinate spray supplement, and water to make 50 gallons was always lethal. A formula three-fourths as strong was lethal in 75 percent of the tests; one-half as strong, in 25 percent.

DISCUSSION

It is generally accepted that the toxicity of chemical agents to micro-organisms varies greatly with environmental conditions. This fact contributes much to the difficulty of making exact comparisons of the relative toxicity of a chemical agent to different test organisms, especially when the latter differ widely in their environmental requirements. In the present work substantial modifications of the basic methods (1, 19, 23), which were devised for use with bacteria pathogenic on man, were necessary to adapt the technique to a test organism pathogenic on plants. Consequently, the results are not strictly comparable with those which have been obtained by the Rideal-Walker method and its modifications on the germicidal efficiency of chemical agents against bacteria that cause disease in man. It has, therefore, seemed unnecessary to follow the Rideal-Walker and similar methods in expressing results in terms of phenol coefficients. Since the major purpose of the present work was to compare the toxic effects of various chemical agents to a single test organism under conditions suited to its environmental requirements, it has seemed more useful to express the results in terms of presence or absence of growth after given medication periods at stated concentrations of the test materials, using molar solutions if feasible and showing for each concentration the percent of the tests in which no growth occurred. The use of solutions based on molecular concentration has important advantages that have long been recognized in work on the toxic effects of chemical agents on fungi (6, 7, 29), seed plants (10), and bacteria (18).

Thomas (25) computed for each time period of medication used in his work the ratio of the lowest concentration of the test material that stopped growth to the lowest concentration of phenol that stopped growth, and took the average of these as the phenol coefficient. Expression of such results in terms of coefficients that relate them to a standard appears to be useful if the major purpose is, as in the work of Thomas, to compare the relative germicidal efficiency of various chemical agents to a group of organisms. Obviously, however, such coefficients are strictly comparable only with others determined by the same technique.

The data in table 1 show that for most compounds tested the lowest concentration which prevented growth varied by one to several dilutions in successive experiments with the same material. Such variations in responses of bacteria to techniques of the type here employed are to be expected, and are in accord with the results obtained by Esty and Williams (8) in studies of thermal death times. This variability adds to the difficulty of accurate expression of results of toxicological studies in terms of coefficients.

Cognizance should be taken of the fact that such laboratory studies as are here reported are necessarily conducted under highly artificial conditions, and do not take into account some of the most important factors that determine the bactericidal efficiency of a material for use in the field, e. g., its resistance to removal by meteoric water. Furthermore, the time periods of medication, 5 and 10 minutes, were chosen primarily because of their extensive use in toxicological studies, rather than as paralleling natural conditions. The results, therefore, bear on the potentialities of materials as field bactericides only insofar as they reveal their comparative toxicity under the experimental conditions.

The data in table 1 show that, under the conditions of these experiments, cupric sulphate and zinc chloride, both of which are used in formulae employed in fire-blight control, are among the least toxic of the chemicals tested.

All the mercury compounds tested were very highly toxic to *Erwinia amylovora,* mercuric cyanide and ethyl mercuric chloride being, however, distinctly less toxic than mercuric chloride, mercurous chloride, and mercuric oxide.

Thomas (25) found mercuric chloride and ethyl mercuric chloride to be highly toxic to *Erwinia amylovora.* Mercuric chloride and mercuric cyanide have long been used as valuable disinfecting agents for treatment of wounds and tools in the excision of diseased parts of plants affected by fire blight (21).

The high toxicity of its compounds to *Erwinia amylovora* seems to make mercury an attractive material for further study in relation to its possible adaptations for increased use in fire-blight control.

Of the orchard sprays tested, lime-sulphur was notable for its low toxicity to the fire blight organism. Field experiments have supplemented the laboratory data in indicating that this fungicide, as commonly used in apple spraying, has little or no value against fire blight. The fire blight organism was easily and repeatedly isolated by plating from spray residues from uninfected leaves on severely blighted trees which had been sprayed 1 to 2 days previously with lime-sulphur, 1 gallon in 50, and lead arsenate, 1 pound in 50 gallons of spray. Lime-sulphur is widely used in often-repeated applications against fungus diseases of apple during a period when a spray suitably toxic to *Erwinia amylovora* might be of substantial value in fire-blight control.

Bordeaux mixture and the zinc sulphate-lime spray were lethal to the fire-blight bacteria at less than the concentrations commonly recommended for orchard use. Lee (15) concluded that bordeaux is valueless against *Phytomonas citri*, unless lime is used in excess, and apparently attributes the toxicity of bordeaux to this organism to the calcium hydroxide in solution. McCown (16) reported that, in his laboratory tests, "neutral" bordeaux was almost as effective as alka-

A METHOD FOR ESTIMATING POPULATIONS OF LARVAE OF THE JAPANESE BEETLE IN THE FIELD [1]

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INTRODUCTION

In developing chemical and mechanical methods for controlling the subterranean larvae of the Japanese beetle (*Popillia japonica* Newman) in the field, it is necessary to determine the density of the larval population from time to time to obtain information on the effectiveness of the different treatments. As the third-instar larva of this insect, a white grub about 1 inch long resembling the native white grubs, is easily seen in most soils, it is possible to determine definitely the number of larvae in a given area by loosening all the soil to a depth of 12 inches with a mattock in the fall and spring and counting the larvae. This procedure is not practical, however, when the area is large, because of the enormous amount of labor involved. Moreover, it is not satisfactory in any soil that is to be examined subsequently to determine the effect of an insecticidal treatment, because many of the larvae are killed or injured mechanically during the operation, some migrate to adjacent undisturbed areas, the cover crop, if present, is injured or destroyed, and the effect of the mechanical and chemical treatments in loosened soil is often different from that in soil which has not been disturbed. It was therefore necessary to develop a method of examining small portions of a given area by which larval populations could be estimated without disturbing all the soil.

EXPERIMENTAL PROCEDURE

In October 1934 four plots, each 50 by 50 feet, were selected at Jacobstown, N. J., in a heavily infested locality for a study of methods of sampling. The boundaries of these plots were carefully marked and the change in elevation in inches was determined with a transit theodolite. The vegetation was noted and the position of all prominent plants recorded. Each plot was divided into 1-square-foot sections, and the number of larvae in each square foot was determined by the procedure described above. These data were recorded in their proper positions on charts drawn to a scale of 1 inch to a foot for use in estimating the average populations by different methods.

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DESCRIPTION OF PLOTS

The topography of the experimental plots is shown in figure 1. Plot 1 was in the lowest section of a pasture. The ground was practically level and was uniformly covered with short pasture grasses. The soil was a heavy clay, slightly sandy in spots, and poorly drained. Plot 2, in the same pasture, was sloping and sandy, well drained, and uniformly covered with short pasture grasses. Plots 3 and 4 were practically level cornfields, with a light, well-drained, loamy soil. Corn was planted at intervals of 4 feet over these plots, except in a strip about 5 feet wide on one edge of plot 4, where tomatoes were growing.
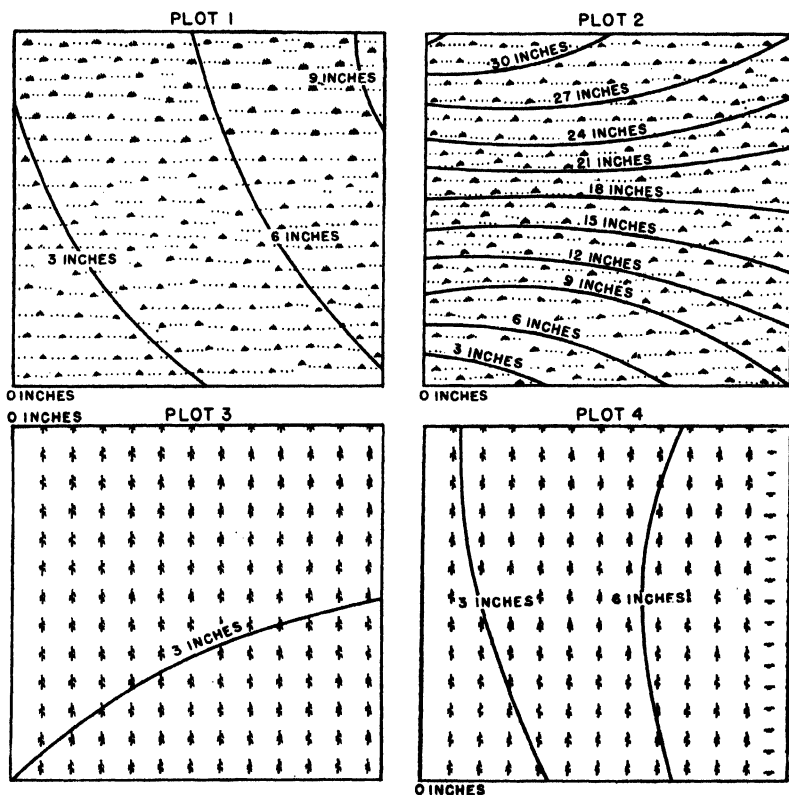
FIGURE 1.—Contour maps of the plots used in estimating populations of Japanese beetle larvae.

LARVAL POPULATION IN THE DIFFERENT PLOTS

The larval populations in the different plots were as follows: Plot 1, 47,866; plot 2, 23,044; plot 3, 12,886; plot 4, 6,909. The larvae were not uniformly distributed over the plots but were more dense in certain sections, as is shown in the dot diagram (fig. 2) for plot 2. The density of the population in the plot ranged from 0 to 46 larvae per square foot. It was not possible to explain this variation by the slight changes in the vegetation or in the elevation of the

land. Such variation of the population in the field is a normal phenomenon.

The numbers of larvae found in each of the 2,500 sections in the different plots were arranged in progressive order and grouped according to the frequency of occurrence. The results are presented graphically in figure 3. It was found that the greater the population in the plot the wider was the variation in the number per square foot and the less frequent was the occurrence of numbers in close proximity to the average. In plot 1, where the population was 47,866 larvae, the number per square foot ranged from 1 to 52,
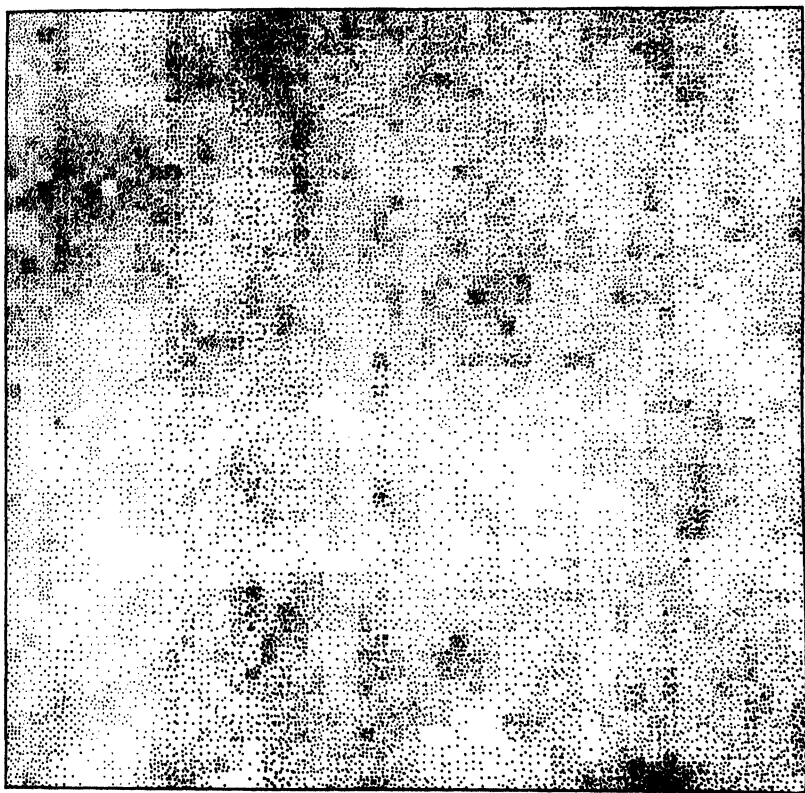
FIGURE 2.—Dot diagram of the distribution of Japanese beetle larvae in plot 2, which was covered with pasture grasses. Each dot indicates one larva.

and 435 cases of the 2,500 were within ± 1 larva of the average, but in plot 4, where the population was 6,909, the variation was from 0 to 20 larvae, and 1,235 cases were within ± 1 larva of the average.

It will be observed (fig. 3) that, with the lightest infestation where many zeros were found, the frequency curve assumed a moderately asymmetrical form. It is to be expected that, when on one side of the greatest frequency the limit is zero and on the other side there is no definite limit, the distribution will be skewed in those

cases where no larvae occur in a large proportion of the units. As the infestation per unit of measurement increased, the zero limit became further removed from the point of greatest frequency, with the result that in the highest infestation the distribution approached the normal symmetrical form. As populations such as those represented in plots 2, 3, and 4 are most frequently encountered in the
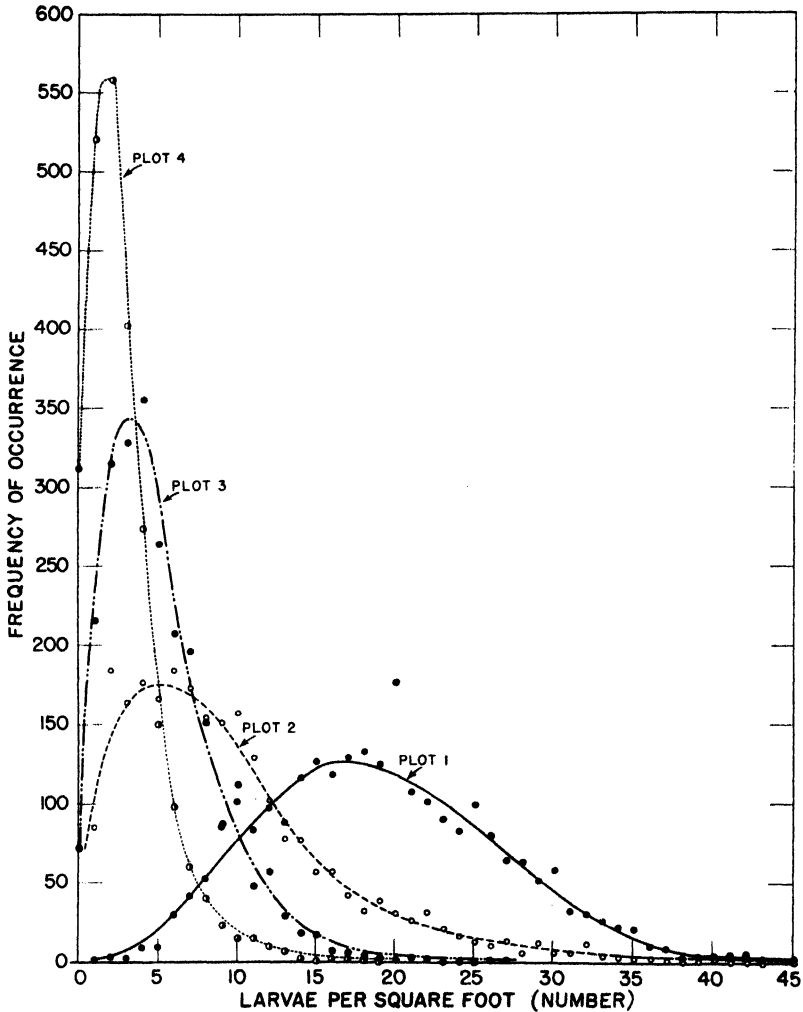
FIGURE 3.—Distribution of larvae of the Japanese beetle in the four plots.

field, it is apparent that the asymmetrical frequency distribution is the most common form in infestations of Japanese beetle larvae.

DETERMINATION OF SIZE OF UNITS TO BE SAMPLED

As the survey charts showed the exact number of larvae in each square foot of each plot, it was possible to combine adjacent 1-square-

foot blocks to form units of any desired size or shape. Units of 1 by 1 foot, 1 by 2 feet, 2 by 2 feet, 1 by 4 feet, 3 by 3 feet, 1 by 9 feet, 1 by 50 feet, and 2 by 50 feet were tried. In the case of the 1- by 4-foot units the width of the rectangles was taken first along the width of the field and then along the length to determine whether the results were significantly changed by modifying the position of these units. The numbers of whole units of these different sizes which could be fitted into a 50- by 50-foot plot were, respectively, 2,500, 1,250, 625, 624, 256, 275, 50, and 25. The average number of larvae and its standard error and the standard deviation, or the error of a single observation, were determined for the different units and are given in table 1. To permit comparison of the errors obtained with the different units, the errors are expressed as relative standard errors, obtained by calculating the standard errors as percentages of the averages.

TABLE 1.—*Statistical constants of larval populations obtained from sampling the entire area of four 2,500-square-foot plots when unit areas of different sizes were used*

Plot no.	Larvae in plot	Size of unit area	Larvae in unit area sampled		
			Mean	Standard deviation	Relative standard error of mean
	Number	*Feet*	*Number*	*Number*	*Percent*
1	47,866	1 by 1	19.15± 0.15	7.44	0.78
		1 by 2	38.30± .38	13.31	.99
		1 by 4 [1]	75.98± 1.00	24.52	1.32
		1 by 4 [2]	76.24± .97	23.81	1.27
		2 by 2	76.59± .97	24.26	1.27
		1 by 9	167.01± 3.24	51.24	1.94
		3 by 3	169.34± 3.34	53.28	1.97
		1 by 50	957.40±33.20	232.43	3.47
		2 by 50	1,914.16±93.60	458.54	4.89
2	23,044	1 by 1	9.22± .14	6.87	1.52
		1 by 2	18.43± .36	12.78	1.95
		1 by 4 [1]	36.68± .97	23.65	2.64
		1 by 4 [2]	36.15± .95	23.22	2.63
		2 by 2	36.87± .97	24.20	2.63
		1 by 9	78.57± 2.59	40.91	3.30
		3 by 3	85.63± 3.02	48.17	3.53
		1 by 50	460.68±13.40	93.81	2.91
		2 by 50	921.56±41.77	204.64	4.53
3	12,886	1 by 1	5.15± .07	3.53	1.36
		1 by 2	10.31± .18	6.20	1.75
		1 by 4 [1]	20.30± .43	10.50	2.12
		1 by 4 [2]	20.07± .43	10.50	2.14
		2 by 2	20.62± .42	10.50	2.04
		1 by 9	45.37± 1.29	20.44	2.84
		3 by 3	44.54± 1.34	21.35	3.01
		1 by 50	257.72± 9.71	67.98	3.77
		2 by 50	515.44±26.73	130.96	5.19
4	6,909	1 by 1	2.76± .05	2.43	1.81
		1 by 2	5.52± .12	4.16	2.17
		1 by 4 [1]	11.25± .29	7.05	2.58
		1 by 4 [2]	10.91± .31	7.58	2.84
		2 by 2	11.05± .30	7.61	2.71
		1 by 9	24.03± .92	14.47	3.83
		3 by 3	25.04± .92	14.70	3.67
		1 by 50	138.38± 3.92	27.44	2.83
		2 by 50	276.36±11.16	54.68	4.04

[1] Width taken along width of field.
[2] Width taken along length of field.

DETERMINED FROM STANDARD DEVIATION FROM THE MEAN

The standard deviation (σ) is the constant usually employed to measure the degree of dispersion of the variates about the average, or mean, of the group. Theoretically about 68 percent of the values should lie within a range of 1 standard deviation above and below the mean. To determine the reliability of this measure of dispersion for the different units used in this study, the percentage of the samples in each unit which was within this range was calculated for the plot of heaviest infestation. It was found that 69 percent of the 1- by 1-foot units were within ± 1 standard deviation of the average. The results with the other units were as follows: 1 by 2 feet, 67 percent; 2 by 2 feet, 64 percent; 3 by 3 feet, 62 percent; 1 by 50 feet, 58 percent; 2 by 50 feet, 56 percent. It is apparent that the standard deviation is an accurate measure of the degree of dispersion when a large number of samples are involved, as in the 1- by 1-foot units, but that it becomes less accurate as the size of the unit is increased, and the number required to cover a given area is decreased. With the larger units the standard deviation indicated a smaller dispersion than actually occurred.

DETERMINED FROM THE STANDARD ERROR OF THE MEAN

When the relative standard errors of the means of the different units are compared, it is apparent that the 1- by 1-foot unit is the most accurate for estimating the number of larvae. As the size of the unit is increased, the relative standard error of the mean becomes larger. With the 1- by 50-foot and the 2- by 50-foot units the relative standard errors do not appear to be correlated with the density of the larval population. The relative standard errors of the small units become greater with the decrease in the number of larvae per unit. It is apparent that when many larvae are present the average population per unit of measurement can be estimated more accurately than when only a few larvae are in the plot.

DETERMINED FROM CONCENTRATION AROUND MEDIAN VALUE

Before this study was undertaken, it was the general opinion that larval populations could be estimated more accurately with square-yard units than with square-foot units. It can be demonstrated with the heaviest infestation, in plot 1, that square-foot units give more accurate measurements because they are grouped more closely about the median value than are the larger units. When the number of larvae per unit is plotted against the frequency of occurrence for units of 1 by 1, 1 by 2, 2 by 2, and 3 by 3 feet, as in figure 4, it will be observed that the values for the 1- by 1-foot units are closely grouped about the median value. As the size of the unit is increased the centralizing tendency gradually disappears, until with the 3- by 3-foot units the values are scattered almost uniformly along the base line. There is no centralizing tendency with the 1- by 50-foot and the 2- by 50-foot units. It is obvious that an average can be estimated most accurately with units that arrange themselves close to the median.
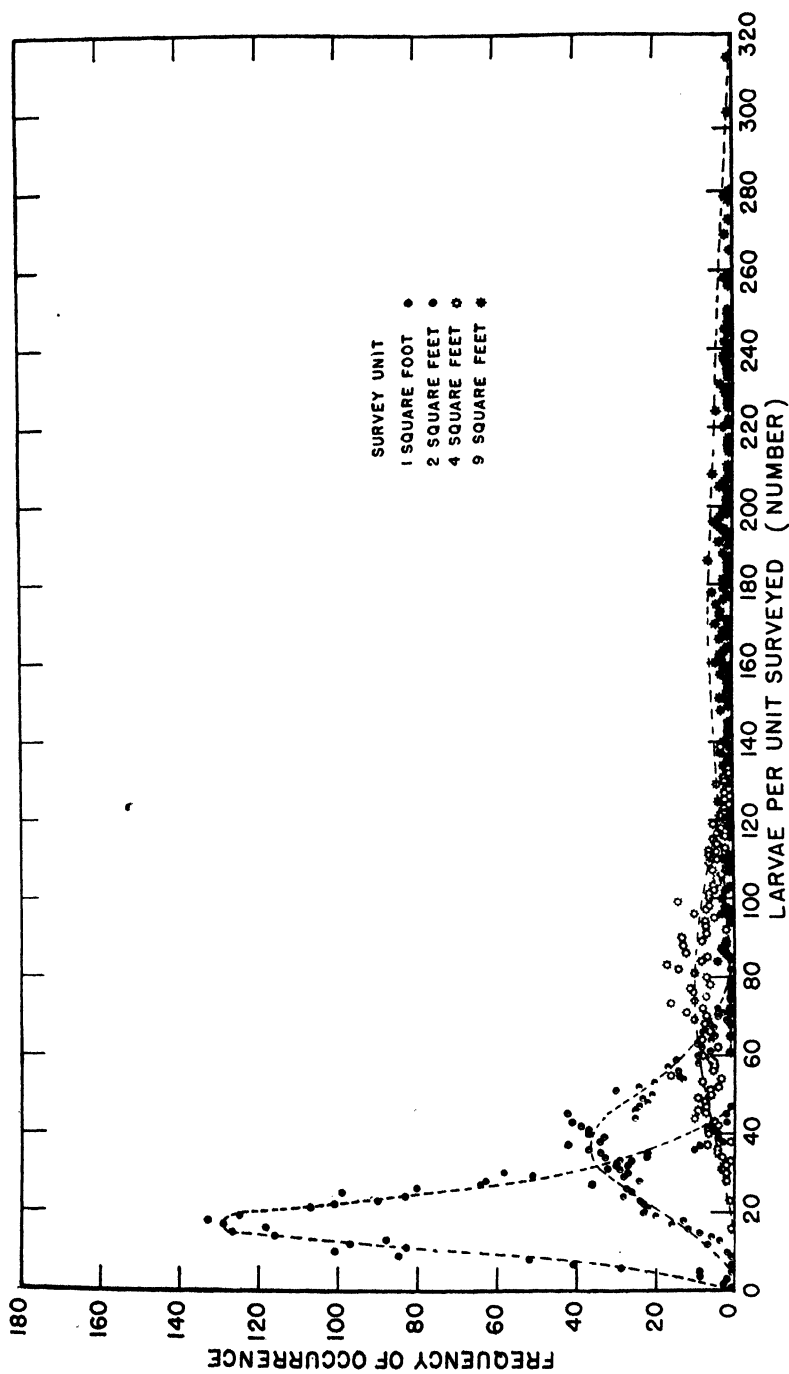
FIGURE 4.—Frequency distributions of the Japanese beetle larvae when grouped in units of different sizes.

EFFECT OF SHAPE OF UNITS

The data in table 1 show that the 1- by 4-foot units gave approximately the same results as the 2- by 2-foot units and that the results with the 1- by 9-foot units were about the same as those with the 3- by 3-foot units. It may be concluded, therefore, that the size, and not the shape, of the units is the determining factor.

CONCLUSION AS TO BEST SIZE OF UNIT

It may be concluded, from a consideration of the standard deviations, the standard errors of the means, and the concentration of the units about the median, that the 1- by 1-foot unit, which is the smallest practical unit for digging in the field, is the most dependable for estimating larval populations in the field.

DETERMINATION OF PERCENTAGE OF AREA TO BE SAMPLED.

DETERMINED FROM STANDARD ERRORS OF THE MEANS

If the standard deviation [2] as determined for the whole plot with any of the above-mentioned units is assumed to be a constant for that unit in the plot, it is possible to estimate the standard errors of samples covering definite percentages of the plot by substituting the

different values for n in the equation $\sigma = \dfrac{\sigma_x}{\sqrt{n-1}}$

For example, if an entire plot was considered in 1- by 1-foot units, n would be 2,500, but if 1 percent of the plot was used, n would be 25. If the plot was taken in 3- by 3-foot units, n would be 256. The standard errors of the means were determined when different percentages of the plots were considered to be covered uniformly with units of the different sizes, and expressed as "relative standard errors" to permit comparison of the errors obtained with the different units. These data are shown graphically in figure 5.

It will be noted from figure 5 that with each unit the relative standard error increases rapidly when the percentage of the area sampled becomes small. The beginning of this rapid increase depends not only on the size of the unit but on the density of the larval population. If it is desired to estimate the average population of a unit area from any of the four plots within a relative standard error of approximately 20 percent, it will be necessary to take about 0.9 percent of the area in 1- by 1-foot units (a), 1 percent in 1- by 2-foot units (b), 2 percent in 2- by 2-foot units (c), 4 percent in 3- by 3-foot units (d), 6 percent in 1- by 50-foot units (e), and 10 percent in 2- by 50-foot units (f). In other words, if 10 percent of the area used in the 2- by 50-foot units was divided into 1- by 1-foot units and distributed at random over the plots, the relative standard error would be reduced from almost 20 percent to less than 6 percent.

EFFECT OF LARVAL POPULATION

As previously stated, the relative standard error is affected by the density of the larval population. When the plots were divided into 1- by 1-foot units, the relative standard error for plot 1 was

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[2] Computed by the formula $\sigma_x = \sqrt{\dfrac{\Sigma x^2}{n}}$.
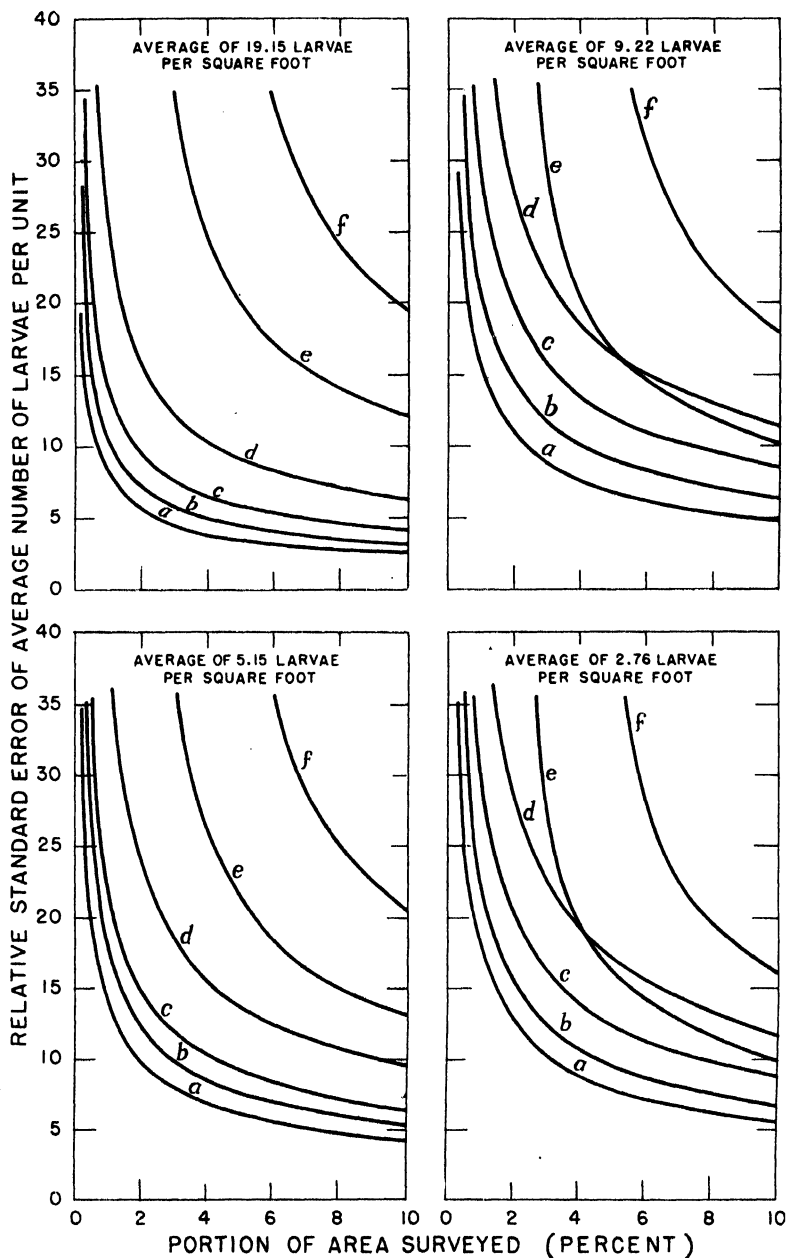
FIGURE 5.—Relative standard error of the mean number of Japanese beetle larvae in the soil when different units were used in making the survey and different percentages of the plot were covered by the sample: *a*, 1 by 1 foot; *b*, 1 by 2 feet; *c*, 2 by 2 feet; *d*, 3 by 3 feet; *e*, 1 by 50 feet; *f*, 2 by 50 feet.

0.78 percent with, an average of 19.15 larvae and for plot 4, 1.81 percent with an average of 2.76 larvae (table 1); when 1 percent of these plots was taken in these units, the relative standard errors were, respectively, 7.92 and 17.98 percent (fig. 5). There seemed to be a straight-line relationship between the relative standard error and the larval population. The lines showing the relationship for the 1- by 1-foot units when different percentages of all the plots were surveyed were determined by the method of least squares, and the results are shown graphically in figure 6. It may be predicted, from the projections of the lines to the right beyond the points of actual determination (shown in broken lines in the figure), that only a small number of 1- by 1-foot units would be required to estimate dense populations ranging from 30 to 40 larvae per square foot. However, since the average number of larvae encountered in most control investigations ranges from 1 to 10 per square foot, and since it is desirable to estimate the average within a possible error of 20 percent, it was decided that at least 1 percent of the area in 1- by 1-foot units should be used in estimating populations of the larvae of the Japanese beetle.
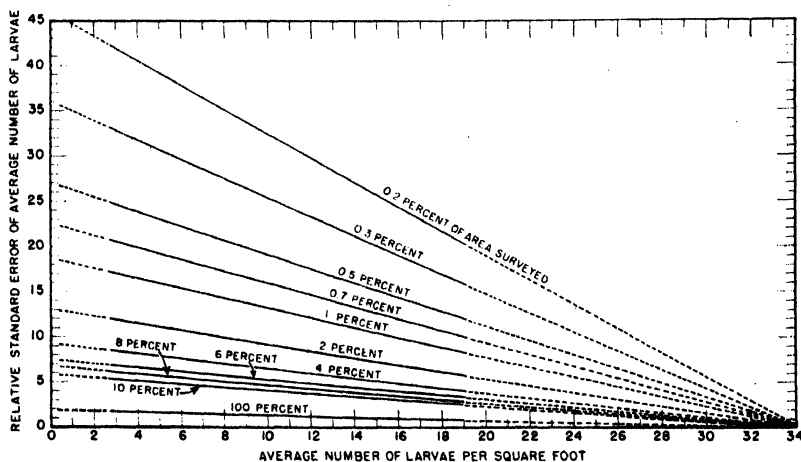
FIGURE 6.—Relation between the relative standard error of the mean number of larvae and the larval population when different percentages of the area were sampled in 1- by 1-foot units.

When the size of the plot is less than 2,500 square feet, it is suggested that a minimum of 25 units be used in the sample. The method of obtaining samples from large areas will be governed to a large extent by the nature of the problem, the local conditions, and the facilities for conducting the work. It is suggested that, when the larval population is to be estimated in an area covering several acres, plots covering approximately 2,500 square feet each be laid out, with at least one plot in a representative part of each acre. After the larval population in each of these representative plots has been estimated accurately, it is possible to estimate with a minimum of labor the population of the whole area and to determine the significance of the difference between different sections.

RELIABILITY OF ESTIMATING LARVAL POPULATIONS FROM
1-SQUARE-FOOT DIGGINGS

In any sampling procedure it is assumed that there is a large "universe" of uniform conditions, in that the individual units vary among themselves in response to the same causes and with about the same variability. The units in a sample should be so selected that the chances of a high value being followed by another high value will be the same as of a low or median value being followed by a high value. When diggings are being made to estimate the number of larvae of the Japanese beetle, there is a tendency to leave those sections where few larvae are found and to continue in those sections where several larvae are found in each digging. When the units of a sample are obtained in this manner, the sample cannot be considered as having been taken at random and the results will not be an accurate estimate of the true average. To eliminate the possibility of biased sampling, it was decided to take the units of a sample at definite intervals over the whole area. When 1 percent of a plot containing 2,500 or more square feet was sampled, the 1-square-foot diggings were made at intervals of 10 feet.

The reliability of this method of sampling was tested by determining the number of samples taken from the four experimental plots which lay outside a specified range from the true average. The average populations of larvae in these plots, as determined from the whole areas, were 19.15, 9.22, 5.15, and 2.76 per square foot (table 1). As the variability of the mean is inversely proportional to the square root of the number of units in the sample, the errors derived from 1 percent of the plots are larger than those given in table 1, which were derived from the whole plot. The standard errors derived from 1 percent of the plots were 1.51, 1.40, 0.71, and 0.49, respectively. One hundred samples, each consisting of twenty-five 1- by 1-foot units, were taken at 10-foot intervals in each plot and the average of each sample was determined. The percentage of cases in which the estimated average was more than 0.5, 1, 1.5, 2, etc., times the standard error of the true average is given in table 2. When the true average was 19.15, 38 percent of the estimated averages differed from the true average by more than 0.5 times the standard error, and in only 1 percent was the variation more than two times the standard error. The variations in the extent to which the estimated values varied from the true averages in the different plots are probably not significant.

The general probability that the estimated average departs from the true value of the universe by more than the stated multiple of the standard error was determined by averaging the departures noted for the different plots. These data are given in the last column of table 2 and presented graphically in figure 7.

It was found that with 1 standard error an average reliability of 75.5 percent was obtained; that is, there were 24.5 chances out of 100 that the true average would not come within the range covered by the estimated average ± 1 standard error. The reliability was 96.5 percent when the standard error was multiplied by 2, thus making the chances rather remote that the true average would not come within the range covered by two times the standard error.

TABLE 2.—*Probability that an average computed from 1-square-foot diggings, taken at intervals of 10 feet with a minimum of 25 diggings, lies farther from the true average than the stated times the computed standard error, for different larval populations in the soil*

Number of times standard error	Probability of estimated average lying farther from true average than stated number of times standard error when true average was—				
	19. 15±1. 51	9. 22±1. 40	5. 15±0. 71	2. 76±0. 49	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
0	100	100	100	100	100. 00
0.5	38	54	46	61	49. 75
1	11	24	24	39	24. 50
1.5	3	10	7	16	9. 00
2	1	5	3	5	3. 50
2.5	0	0	2	1	. 75
3	0	0	1	0	. 25
3.5	0	0	1	0	. 25
4	0	0	0	0	. 00
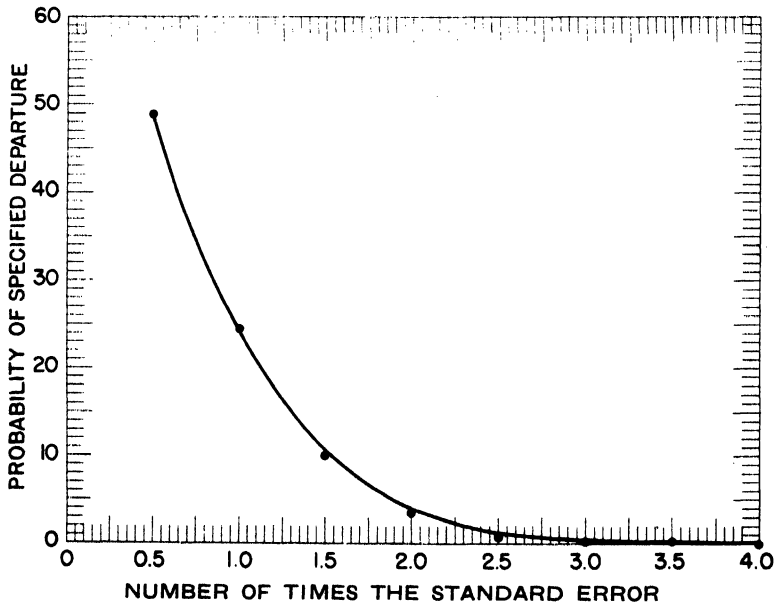
FIGURE 7.—General probability that an average computed from a sample lies farther from the true value than the stated number of times the standard error for populations averaging between 2.76 and 19.15 larvae per square foot when a minimum of 25 units constitutes the sample.

When it is desired to estimate a larval population with a high degree of accuracy, it is suggested that the area be sampled sufficiently to reduce the relative standard error to less than 10 percent. Thus, if 1 percent of a plot is sampled and the average population is estimated as 10 larvae per square foot, it will be seen from figure 6 that the average relative error for this density of population and proportion of the area would be approximately 14 percent, indicating that the sampling should be more extensive. In this case it would be desirable to continue the sampling until 2 percent of the plot had

been examined. If the sample in the example covered 2 percent of the area, and if the standard error of the final estimate was multiplied by 2, it would be expected that the estimated average would have a reliability of 96.5 percent and that twice the relative standard error would be less than 20 percent.

SUMMARY AND CONCLUSIONS

A method has been developed for estimating the population of larvae of the Japanese beetle in the field. The number of larvae in each square foot of four 2,500-square-foot plots was determined, and the results were used in estimating the true averages in each plot by different methods.

The 1-square-foot unit was found to be the most accurate for estimating the population. As the size of the sampling unit was increased, the error became progressively larger. The size, and not the shape, of the sampling unit was the modifying factor.

The error of the estimate is influenced to some extent by the density of the population. It is possible to estimate a dense population more accurately than a sparse population.

It is recommended that in estimating the larval population a minimum of 25 units of 1 square foot uniformly distributed constitute a sample from plots containing less than 2,500 square feet, and in larger areas that the units be spaced not more than 10 feet apart. The larval populations can be estimated most accurately with a minimum of labor in large areas covering several acres by estimating the number of larvae in 2,500 1-square-foot plots placed in representative portions.

By proper sampling it was found to be possible to obtain a reliable estimate of the larval population in a given area.

ALFALFA DWARF, A VIRUS DISEASE TRANSMISSIBLE BY GRAFTING[1]

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INTRODUCTION

Alfalfa dwarf was reported and described for the first time by the writer *(11)* [2] in 1931 as a disease of unknown etiology. In a later paper *(12)* he reported that temperature, soil moisture, soil fertility, and the cutting of plants in an immature stage were not causal factors, nor was the disease due to an excess or deficiency of boron, manganese, strontium, bromine, titanium, iodine, aluminum, copper, or zinc. These conclusions are confirmed by the following field observations: (1) Dwarf does not attack plants uniformly in certain areas; (2) it is most destructive where growth is best; and (3) soils on which the disease is most destructive grow luxuriant alfalfa at each reseeding.

Concomitant with the studies which showed that dwarf is not due to these environmental and cultural factors, experiments were conducted to determine whether the disease is due to some micro-organism or to a virus. Since this project has now been discontinued, the results of these experiments are reported in this paper.

METHODS

The alfalfa *(Medicago sativa* L.) plants used in these experiments, unless otherwise stated, were of the Arizona Common or Hairy Peruvian varieties, and they were grown in pots, or in small basins on land that had never grown alfalfa, or in the field. In most cases they were less than a year old and, as nearly as could be determined, free of dwarf or other root diseases. After the plants were inoculated with bacteria, by grafting or otherwise, as hereinafter described, they were replanted and allowed to grow for several months before they were examined. In some of the experiments plants grown in the field were inoculated without being removed from the soil.

Unless otherwise stated, the work with potted plants was done in the open on the campus of the University of California, Riverside, Calif., nearly a mile from the nearest alfalfa field, and pots of different sizes as well as 16-gallon garbage cans were used.

When uninfested soil was desired, 4 to 6 inches of surface soil was removed from an area on the mountainside, where no cultivated crop had ever grown, and the subsoil thus exposed was used. The fact that no infection from this subsoil was ever observed in the controls attested to the safety of this procedure. Soil was taken from about diseased plants to determine the presence or absence of the causal agent in it.

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Many plants were set in basins made on land that had never grown alfalfa. These basins were usually 4 by 6 feet and were constructed by removing about 6 inches of the surface soil and placing it about the margin of the area in order to retain the irrigation water and thus permit independent watering of the basins. No evidence of the disease spreading from one basin to another was ever observed.

ISOLATIONS AND INOCULATIONS

Isolations were made from nearly 1,000 dwarfed alfalfa plants, having all stages of disease, and from all parts of the plants and at every season of the year. Both naturally infected plants and those having the disease as a result of grafting were studied. A large number of media were tried, including several liquid media as well as many of the commonly used agars, with different amounts and sources of carbon and nitrogen and various hydrogen-ion concentrations. The isolations were made in different ways, including poured plates and tissue plantings. Anaerobic conditions were supplied in some instances. In a few cases a sterile capillary glass tube was stirred about in a plugged duct under aseptic conditions and the tube was wiped over the surface of an agar plate. Some of the contents of the plugged ducts were drawn into the tube and discharged on the surface of agar in plates. The manipulation of the glass tube was accomplished by a slight modification of the method described by Roberts (*10*) for picking up single spores. Sterile distilled water was drawn through segments of diseased roots by means of suction under aseptic conditions, and the water was plated on agar. Numerous other measures were tried in an attempt to isolate a causal micro-organism from the diseased tissue, but it is not necessary to discuss these in detail. Suffice it to say that these isolations failed to yield with any degree of consistency any one organism, or one that was capable of causing infection under the experimental conditions provided. Bacteria did appear in about 75 percent of the plates, but similar-appearing organisms were often obtained from control isolations from healthy tissue. In by far the larger number of cases the isolations were made from the wood of the roots, as this was the tissue most conspicuously affected. A high percentage of the plantings from the wood of the root after the bark was peeled back aseptically gave bacteria. Poured plates commonly remained sterile or gave only one or a few scattered colonies, often no two being alike. A single planting from a diseased root often gave two or more species of bacteria, indicating that the alfalfa roots affected with dwarf soon were invaded with secondary bacteria. Fungi also were sometimes present.

Inoculations were made only with the bacteria appearing most consistently in the isolations, some of them being used several times. In a number of instances several or all of the bacteria obtained on a set of plates were washed into a beaker with sterile distilled water and used as inoculum. Plants that were wounded, not only by breaking the roots when they were removed from the soil but also by cutting the taproot beneath the suspension, were stood in the suspension for 2 hours or longer and then replanted. In some cases the plants were inoculated in place by (1) pricking a broth culture into the taproots or stems, (2) cutting off the stems with a razor and, as quickly as

possible, placing some of the broth culture on the fresh wounds with a pipette dropper, or (3) dipping pieces of healthy wood into bacteria on plates or slants and inserting them into wounds in taproots of healthy plants. Small pieces of diseased roots, cut as nearly aseptically as possible, were dropped into flasks of nutrient broth or sterile distilled water, and after 24 hours or longer healthy plants were stood in the culture for a time and then planted. In other instances pieces of cotton were first dipped into the broth culture and then forced into the taproot of a healthy plant and allowed to remain. Not a single plant inoculated with bacteria became diseased. The results of the isolations and inoculations seem to justify the conclusion that alfalfa dwarf is not caused by a micro-organism that can be handled by the methods generally used in bacterial plant pathology. The conclusion that dwarf is not caused by a bacterium is strengthened by experiments dealing with the method by which the disease is spread, which will be discussed later in this paper.

TRANSMISSION EXPERIMENTS WITH VARIOUS AGENCIES

SOIL

The first experiments were planned to determine whether dwarf is soil-borne. Soil from about the roots of badly diseased plants from different sources was placed in pots, in 16-gallon cans, or in basins constructed as described under Methods. Healthy plants or seeds were planted in this soil and allowed to grow in the open for various periods. Controls consisting of healthy plants or seeds planted in mountain soil were run in most cases. Sometimes part of the soil was sterilized with formaldehyde (250 cc of 40-percent formaldehyde in 1 gallon of water) before planting, the unsterilized part being used as a control. The data obtained are given in table 1.

TABLE 1.—*Effect of setting healthy alfalfa plants in soil from about diseased plants*

Date plants set out	Date final notes taken	Source of soil	Soil treatment	Plants showing indicated condition at end of experiment	
				Healthy	Diseased
				Number	*Number*
Apr. 4, 1929	Oct. 18, 1930	Chino	Not sterilized	4	0
		do	do	24	0
		Riverside	do	33	0
Oct. 30, 1929	July 10, 1933	do	Sterilized	8	0
		Arlington	Not sterilized	25	0
		do	Sterilized	50	0
Oct. 30, 1930	do	Riverside	Not sterilized	9	0
Sept. 8, 1931	do	do	do	22	0
		do	do	18	4
		do	do	22	1
Sept. 12, 1932	Aug. 6, 1934	Original mountain soil in basin used as control for other 2 experiments of same date.	do	22	1

Only in the experiment set up on September 12, 1932, did any infection develop in the soil in which dwarfed plants had grown. In this experiment the soil in two basins, each about 4 feet square, was removed and replaced to a depth of 1 foot with soil from about badly diseased plants from two different fields. The original soil was left

in a third basin as a control. Twenty-five young healthy plants were set in each basin. At the end of the first year no disease was apparent in any of the basins, but after 2 years 4 out of 22 plants and 1 out of 23 plants were diseased in the two basins in which the soil had been replaced, while 1 diseased plant appeared in the control basin. Since one plant developed the disease through natural infection in the control basin, it is possible, and even probable, that the five infected plants in the other two basins also were infected by natural means and not by soil from about diseased plants.

In addition to these experiments, some evidence that dwarf is not soil-borne was obtained in a study of insect transmission. Approximately 200 healthy plants set under cages in soil that had grown badly dwarfed plants for the 2 preceding years were still healthy at the end of July of the second growing season.

The data given above seem to show fairly conclusively that the causal agent of dwarf does not live, at least to any considerable extent, in soil that is free of affected plant tissue. It also lends strength to the view that dwarf is not caused by an unsuitable soil condition, such as an excess or a lack of certain necessary or toxic chemicals.

DISEASED PLANTS

Since dwarf did not appear to be transmitted through the soil, experiments were designed to determine whether the disease would spread from a diseased to a healthy plant. The results of these experiments are recorded in table 2.

TABLE 2.—*Spread of dwarf from diseased to healthy plants*

Date plants set out	Date final notes taken	Plants inocu-lated	Control plants	Plants infected			
				Inoculated		Control	
				Number	Percent	Number	Percent
		Number	Number				
Dec. 2, 1930	June 9, 1932	10	10	8	80	0	0.0
Feb. 27, 1930	July 3, 1931	20		16	80		
Feb. 17, 1931	Sept. 22, 1931	50	250	13	26	0	.0
Mar. 1, 1934	Sept. 21, 1934	200	200	30	15	1	.5
Sept. 30, 1932	Aug. 20, 1934	48	75	11	23	0	.0
May 16, 1934	May 28, 1935	{ 46	60	16	35	0	.0
		7	� 7	2	29	0	.0

[1] Entire diseased plants were killed by boiling before being planted.

In the first experiment four 16-gallon cans filled with mountain soil in which badly diseased plants had been growing for the 2 preceding years were used. The dwarfed plants still alive were removed from two of the cans, while those in the other two cans were allowed to remain. Five healthy plants were set in each can on December 2, 1930, and by June 9, 1932, four out of five, or 80 percent, of the new plants in each of the two cans from which the dwarfed plants had not been removed were affected with dwarf, whereas no disease had developed in the other two cans. These two sets of cans differed only in the fact that the diseased plants were present in one set. The presence of these diseased plants resulted in the difference between 80-percent infection and none at all, thus suggesting that the causal agent survives only in living tissue.

Further evidence of the rapidity of the spread of dwarf from diseased to healthy plants was obtained by setting diseased and healthy plants alternately in pots, cans, basins, or rows in the field. Comparable controls of healthy plants only were run. In the field or in the basin the plants were set 6 inches apart in the row. As shown in table 2, the percentage of infection obtained varied from 15 to 80 percent. In only one case did a control plant become infected.

Although designed for another purpose, the results of an experiment set up on September 30, 1932, give additional data on the subject under discussion. Forty-eight healthy plants were set on one side of a 3-foot board partition and 52 diseased plants on the other side. The partition kept the tops from intermingling but not the roots. On August 20, 1934, 37 of the 48 originally healthy plants remained healthy and 11, or 23 percent, were diseased. Seventy-five healthy plants growing in a basin nearby as controls remained healthy.

These experiments show that dwarf does spread from plant to plant, but they do not reveal the method by which the causal agent is transferred from diseased to healthy plants.

<div align="center">WATER</div>

Previous experiments (*12*) have shown that the severity of alfalfa dwarf is greatly influenced by soil moisture, the disease being more destructive on soil kept sufficiently wet for vigorous plant growth than on dry soil. Frequently the disease appears first in severe form about standpipes or where water is applied most heavily. This suggests the possibility that the disease is distributed by water.

One experiment designed to throw light on this point and initiated February 17, 1931, consisted in the use of three basins situated at different levels on a mountainside where cultivated crops had never been grown. One hundred young healthy plants each were set in the upper and lower basins, and 50 diseased plants in the center basin. The upper basin was watered independently and served as a control. The lower basin was watered by the overflow from the center basin. No dwarf had developed in either of the end basins when the experiment was discontinued on October 6, 1933.

Alfalfa was grown in two rows, each about 150 feet long, on land where it had not been grown before. Diseased plants from the field were set in the 50 feet of row 1 nearest the irrigation ditch, and seed was sown in the remainder of the row. Row 2 was divided into sections that were planted with seed and diseased plants alternately, so that there were four sections sown with seed and three set with diseased plants. The plants were set in the bottom of furrows 6 inches deep so that the irrigation water would come into contact with the crowns of the plants. The plants grown from seed in the first 15 feet of row 2 nearest the irrigation ditch received water that had not come in contact with the diseased plants; however, the second, third, and fourth lots of healthy plants (grown from seed) in this row received water that had first passed around one, two, and three sections of 25 diseased plants, respectively. At the end of 1 year half as many plants affected with dwarf appeared at the end of row 2 next to the irrigation ditch as at the other end, and the disease had spread to three rows of alfalfa in an adjoining plot that had received no water previously in contact with diseased plants. Regardless of the fact that in

row 2 the water came successively in contact with more diseased plants as it flowed from section to section, in July of the second year little difference was found in the percentage of dwarf in any of the four sections of plants grown from seed. In row 1, out of 248 plants grown from seed, 24, or approximately 10 percent, were diseased. The distribution of the disease in these two rows, together with that in the four adjacent rows, indicates that the water was not the principal agent involved in carrying the causal agent.

Additional evidence that water is not the important agent in distributing dwarf in the field was obtained by preparing a seedbed about 3 feet wide by 100 feet long in such a way that the top of the bed was several inches higher than the furrows in which the irrigation water ran. The elevated seedbed and the furrows were seeded with common alfalfa on March 12, 1930. The plants on the raised bed were able to get sufficient moisture without the water coming in contact with the crowns. Of course the winter rains could not be excluded, but since the bed was elevated and a furrow was present on either side, the water ran over the surface only a very short distance. On November 18, 1932, there were 30 percent of dwarfed plants on the raised bed and 22 percent in the furrows, showing that the disease was equally as serious where the irrigation water did not come in contact with the tops or crowns of the plants as where it did. This was considered important, as dwarf can be detected first in the crowns.

DISEASED TISSUE

Since the disease did not appear to be soil-borne, but was transmissible from plant to plant, it seemed desirable to ascertain whether plants growing in soil in which pieces of diseased tissue were present would take the disease. The results of experiments designed to determine this point are shown in table 3.

Soil from about badly diseased plants was placed in a pot and diseased roots cut in short lengths were added; Hairy Peruvian alfalfa was sown in it on April 4, 1929. On April 28, 1931, out of 17 plants, 9, or 53 percent, had dwarf. Control plants growing in mountain soil remained healthy.

TABLE 3.—*Transmission of dwarf by means of diseased tissue in the soil*

Date plants set out	Date final notes taken	Plants inoculated	Control plants	Plants infected				Part of plant tissue used
				Inoculated		Control		
		Number	*Number*	*Number*	*Percent*	*Number*	*Percent*	
Apr. 4, 1929	Apr. 28, 1931	17	4	9	53	0	0	Roots.
Sept. 12, 1932	Aug. 3, 1934	25	25	0	0	0	0	Stems.
Do	do	25	25	4	16	0	0	Roots.
May 16, 1934	May 28, 1935	14		0	0	0	0	S t e m s a n d roots.

On September 12, 1932, a quantity of stems from badly diseased plants were cut into small pieces and mixed into the surface 2 inches of soil of a 4- by 4-foot basin. A control was prepared in the same manner except that healthy stems were used. Thinly sliced diseased roots were mixed into the soil of a third basin, and a fourth was left

untreated. Each of the four basins was planted with 25 young healthy alfalfa seedlings. On August 3, 1934, no disease was found in the basins to which the stems had been added or in the untreated basin, but in the basin to which the diseased roots had been added there were four diseased plants.

Although too few to be conclusive, these experiments suggest that dwarf may be carried in the soil in pieces of root tissue but probably not in the green stems. Just how the causal agent gets from the segments of diseased roots into the roots of the healthy plants under these conditions is not known, unless it is carried by some soil insect.

On May 16, 1934, 14 healthy plants were set in cans in mountain soil and the roots were surrounded by a quart of diseased root and stem tissue that had been passed through a food grinder. No disease had developed by May 28, 1935. Seemingly every opportunity for infection was offered had there been a causal agent present that was capable of penetrating the roots directly or through wounds.

Since the disease was not carried by the diseased stems in one of the experiments reported above, it seems probable that the disease is not carried in the hay or seed. This is a single experiment, however, and should not be given too much weight, although the results are apparently confirmed by the experiments with stem tissue and juice, which are described later in this paper.

CUTTINGS

Although the stems of diseased alfalfa plants when added to the soil did not transmit dwarf to healthy plants, the possibility that the causal agent is in the stems was not entirely excluded. In order to determine more definitely whether the disease is present in the green tops, the experiments recorded in table 4 were conducted.

Two sets of cuttings were made from stems of dwarfed plants on February 17, 1930. In one set some of the crown tissue was included and in the other only green tissue was used. These cuttings were rooted in sand and later planted in cans of mountain soil. Several cans of the same soil containing 100 healthy plants grown from seed were used as controls to check on possible outside sources of infection. On August 18, 1931, of the plants grown from cuttings from green stems alone, only four had dwarf, four remained healthy, and two had died from some unknown cause, possibly dwarf; whereas of the plants grown from cuttings that included some of the crown tissue, eight had dwarf, one was healthy, and one had died of crown rot. A higher percentage of infection in the latter group suggests that the disease was more consistently present in the bases of the stems and in the crown tissue than in the green stems. No disease developed in any of the controls.

On December 30, 1932, cuttings were made from the upper ends of the stems of plants in an early stage of dwarf, where it seemed very improbable that any of the bacterialike bodies or gum in their vessels would be found, especially as the stems had grown late in the season, when dwarf is inactive. Comparable cuttings were made from healthy stems as controls. The cuttings were rooted in sand and transplanted to soil in cans. On July 20, 1934 the plants were examined, and 15 out of 43, or 35 percent, of those from diseased plants had dwarf. The remainder, as well as the controls, were healthy.

TABLE 4.—*Transmission of dwarf in cuttings from diseased stems*

Date cuttings made	Date final notes taken	Cuttings		Plants infected			
		Healthy control	Dis- eased	From healthy plants		From diseased plants	
		Number	*Number*	*Number*	*Percent*	*Number*	*Percent*
Feb. 17, 1930_____	Aug. 18, 1931_____	{ [1] 50	[2] 10	0	0	4	40
		[1] 50	[2] 10	0	0	8	80
Dec. 30, 1932_____	July 20, 1934_____	50	43	0	0	15	35

[1] Plants grown from seed. [2] Cuttings from green stems alone. [3] Cuttings included some crown tissue.

This experiment, like the one previously described, shows that the causal agent is present in at least part of the green stems of dwarfed plants. All of these cuttings were made during the winter when the dwarf was either entirely inactive or nearly so. Many cuttings were made at other times but none grew, hence only results from cuttings made in the winter are available.

LEAF MUTILATION AND PINPRICK INOCULATION

As already shown, the dwarf disease is present even during the winter in at least a part of the green stems. Attempts were made, therefore, to transmit dwarf by certain more or less standard methods used by investigators of virus diseases.

Two methods of leaf mutilation were tried. In the first, leaves of diseased and then of healthy plants were crushed between the thumb and fingers, and in the second, healthy and diseased leaves and stems were crushed together. One hundred plants were thus treated, but no disease resulted.

In another experiment 12 micro-insect pins, pushed through a piece of cardboard to hold them in position, were used to prick juice from diseased into healthy alfalfa leaves. Terminal leaves of diseased and healthy plants were held in contact with one another and pricked repeatedly. A number of leaves on 27 plants were inoculated in this manner, but no disease developed in any of them.

JUICE

Several attempts were made to transmit dwarf by inserting juice from dwarfed tops and roots into healthy plants. Juice, obtained by macerating the tissue in a food grinder and squeezing it by hand through cheesecloth, was injected into the healthy plants as soon as possible after extraction, each plant usually being subjected to several injections. Controls in which juice from healthy plants or sterile distilled water replaced the juice from diseased plants were prepared in some cases. Cotton dipped into the juice of diseased plants was inserted into the root or the base of the stems of healthy plants and allowed to remain. In other cases juice was injected into healthy roots with a hypodermic needle or applied to freshly cut stems with a pipette dropper. None of the 95 inoculated or the 95 control plants became infected. That dwarf is not transmitted, at least not readily, by means of diseased juice applied mechanically was concluded.

TISSUE INOCULATION

Experiments were conducted to determine whether dwarf could be transmitted by inserting bits of diseased tissue into healthy plants.

For the most part this type of experiment consisted in making an opening into the healthy plant by means of a scalpel, inserting the diseased tissue, and either leaving the wound open or, more often, closing it as well as possible with tape, raffia, paraffin, or tree seal. In many cases the inoculum consisted of a small piece of yellow wood, about 15 to 20 mm long, 1 to 2 mm thick, and 5 to 10 mm wide, taken from a diseased taproot after the bark was removed. Sometimes the tissue was macerated with a mortar and pestle before it was inserted into the wound. Pieces of stems, rootlets, leaves, buds, bark from the taproot, and white wood from diseased roots were tried as sources of inoculum. An attempt was made in many cases to crush the diseased tissue after it had been placed in the wound, so that some of the juice from the diseased tissue actually might be mixed with that of the healthy wood. Comparable controls were run in most cases.

Table 5 shows that out of a total of 1,114 plants inoculated 63 plants, or 6 percent, became infected. Of the 409 controls, only 1, or 0.24 percent, developed the disease. Of 672 plants, infection resulted in 63, or 9 percent, when yellow wood was used as the inoculum; the number infected included the 1 plant inoculated with crushed rootlets. Only in the experiment initiated July 12, 1932, with yellow wood, was there a high percentage of infection, namely, 84 percent. Why such a high percentage of infection was obtained in this experiment is not known. Infection resulted only in those experiments in which yellow wood was used as the inoculum.

TABLE 5.- *Transmission of alfalfa dwarf by inserting diseased tissue into healthy plants*

Inoculum used	Date inoculated	Date final notes taken	Plants inocu-lated	Con-trol plants	Plants infected			
					Inoculated		Control	
			Num-ber	*Num-ber*	*Num-ber*	*Per-cent*	*Num-ber*	*Per-cent*
Yellow xylem	June 7, 1930	Aug. 19, 1931	5	0	0	0	------	------
Do	Aug. 12, 1930	July 3, 1931	10	10	1	10	0	0
Do	Nov. 7, 1930	Nov. 19, 1931	10	0	0	0	0	0
Do	Dec. 5, 1930	Sept. 3, 1931	50	50	15	30	1	2
Do	June 15, 1931	Sept. 6, 1932	25	1 50	0	0	0	0
Green stems	----do	----do	50	--------	0	0	0	0
Leaves	----do	----do	25	--------	0	0	0	0
Crushed yellow xylem	----do	----do	25	--------	3	12	0	0
Crushed bark	----do	----do	25	--------	0	0	0	0
Crushed rootlets	----do	----do	25	--------	1	4	0	0
Yellow xylem	Apr. 8, 1932	June 23, 1933	43	25	0	0	0	0
Do	May 9, 1932	----do	44	25	0	0	0	0
Do	June 17, 1932	----do	45	25	0	0	0	0
Do	July 11, 1932	----do	46	25	1	2	0	0
Do	Aug. 26, 1932	----do	49	25	0	0	0	0
Do	July 12, 1932	Nov. 29, 1932	50	24	42	84	0	0
Green stems	----do	----do	50	25	0	0	0	0
White xylem from diseased plants.	----do	----do	50	25	0	0	0	0
Yellow xylem	Jan. 5, 1933	June 22, 1933	100	25	0	0	0	0
White wood from diseased root.	June 6, 1934	Sept. 21, 1934	34	10	0	0	0	0
White bark from diseased root.	----do	----do	52	35	0	0	0	0
Yellow xylem	----do	----do	145	10	0	0	0	0
Green stems	----do	----do	54	10	0	0	0	0
Terminal leaves	----do	----do	57	10	0	0	0	0
Bark over yellow wood	----do	----do	35	0	0	0	0	0
Total			1, 104	409	63	2 6	1	2 .24

1 These 50 plants served as a control for all of the inoculations made on June 15, 1931.
2 Average percent.

APPROACH GRAFTING

Since grafting has been found to be one of the most successful methods of transmitting virus diseases that are not readily transmitted mechanically, a type of approach grafting was tried. This consisted in cutting away a portion of the bark and wood of the taproots of a diseased and of a healthy plant, placing the wounded surfaces together, and fastening them in that position by means of rubber electric tape or raffia (fig. 1). The plants were then set in

FIGURE 1.—Approach grafting of diseased and healthy alfalfa plants. The larger roots were diseased. The smaller roots were healthy when grafted but were diseased when photographed, although top symptoms were not yet conspicuous. About one-fifth natural size.

TABLE 6.—*Transmission of alfalfa dwarf by approach grafting*

Date grafted	Date final notes taken	Plants grafted	Control plants	Plants infected [1]	
		Number	*Number*	*Number*	*Percent*
May 8, 1930	Aug. 18, 1931	3	0	2	67
Nov. 7, 1930	Nov. 19, 1931	4	0	3	75
July 15, 1931	Sept. 2, 1932	25	0	8	32
Apr. 8, 1932	June 23, 1933	45	25	13	29
May 24, 1932	do	39	25	25	64
June 17, 1932	do	33	25	19	58
July 11, 1932	do	33	25	7	21
Aug. 25, 1932	do	5C	25	8	16
May 25, 1932	do	50	0	7	14
July 15, 1931	Sept. 2, 1932	25	25	8	32
Mar. 1, 1934	Aug. 1, 1934	20	25	3	15
Do	do	23	25	10	43
Do	do	26	25	7	27
Do	do	91	25	28	31
May 16, 1934	May 28, 1935	8	[2] 8	3	38
June 13, 1934	Sept. 17, 1934	35	0	11	31
Total		510	258	162	[3] 37

[1] None of the control plants became infected.
[2] Entire diseased plants were killed by boiling in water and were then grafted to healthy roots.
[3] Average percent.

cans or basins and allowed to grow, usually for several months, before they were examined. The results of a number of such experiments are recorded in table 6. Controls were prepared by grafting two healthy plants. In the experiment set up on May 16, 1934, the control consisted of diseased plants that were killed by being boiled in water before they were grafted on healthy plants. A total of 510 plants were grafted, of which 162, or 32 percent, became diseased. No disease developed in any of the 258 controls.

During the spring and summer of 1932 grafts were made in April, May, June, July, and August, in order to study seasonal influences. In this experiment, as shown in table 6, the highest amount of infection developed in the plants grafted early in the season, namely, in April, May, and June. This was probably due, in part at least, to the fact that earlier in the season the plants recovered more quickly and completely from the shock of transplanting, and possibly also to more rapid and extensive union of the diseased and healthy tissues. On the other hand, the lowest percentage of infection obtained resulted from the inoculations made on May 25, 1932. Although the infection that developed in these 16 experiments averaged only 37 percent, it is evident that the dwarf disease can be transmitted by approach grafting. In many cases no union or only a very poor one was formed between the diseased and healthy plants; in others there was a fair union at the upper parts of the wounds, although usually the amount of tissue grown together was not large. The evidence indicates that a definite union is necessary, at least for a high percentage of infection. In a few instances no union was apparent at the time the plants were examined, and yet transmission had taken place. Apparently only a slight union is necessary, and it is possible that occasionally transmission takes place without a union.

OTHER GRAFTING METHODS

Other methods of budding and grafting were tried in an effort to find a more consistent means of transmitting dwarf. Scions consisting of part crown and part stem tissue and buds taken from the crowns of diseased plants were inserted into the crown branches of healthy plants by the cleft-graft method, but they failed to grow, and no disease resulted.

The results of three other types of grafting are shown in table 7. In the first two experiments a piece of diseased taproot about 3 inches long was grafted on the taproot of a healthy plant about 6 inches below the crown by the ordinary cleft-graft method (fig. 2). The cleft was made in the old root, and, after insertion of the wedge-shaped end of the healthy root, the graft was wrapped tightly with raffia and covered with tree seal. Figure 3 shows that a high percentage of infection was obtained. A union was formed in all but 1 of the 31 plants inoculated in these two experiments, and infection resulted in all but this 1 plant. Roots grafted in this manner formed a union more consistently and made a better union than was done by any other method tried.

On June 15, 1931, the ends of a healthy and of a diseased root were grafted, the end of the diseased root, about 6 inches below the crown, being used as the stock and that of the healthy root as the scion. A considerably lower percentage of infection resulted (35 percent), but

there is no doubt that infection can be obtained by this method. The grafts in the last experiment in table 7 were made in a similar manner except that a slit was made entirely through the diseased root, through

FIGURE 2.—Healthy control alfalfa plant at left. Three plants at right are from same lot of plants as the control, but are dwarfed as a result of grafting a piece of diseased root to their taproot. Grafted June 7, 1934, and photographed September 9, 1934. About one-sixth natural size.

which the healthy root was inserted, so that the two crowns were at a common level. The bark was removed from the healthy root at the point of contact with the diseased one. This was similar to an insert

graft except that the healthy root passed entirely through the diseased root and the cambiums made contact on both sides of the roots. These experiments indicate that dwarf can be transmitted by any method of grafting in which a union is formed.

TABLE 7.—*Transmission of dwarf by other grafting methods*

Date grafted	Date final notes taken	Plants grafted	Control plants	Plants infected [1]	
		Number	*Number*	*Number*	*Percent*
June 7, 1934 [2]	Sept. 18, 1934	21	25	21	100
June 5, 1934 [2]	Sept. 21, 1934	10	10	9	90
June 15, 1931 [3]	Sept. 2, 1932	23	25	8	35
Mar. 1, 1934 [4]	Sept. 21, 1934	18	25	10	56

[1] None of the control plants became infected.
[2] Section of diseased root grafted on the end of a healthy root.
[3] Ends of healthy and diseased roots grafted together.
[4] Healthy root inserted through a slit in the diseased root.
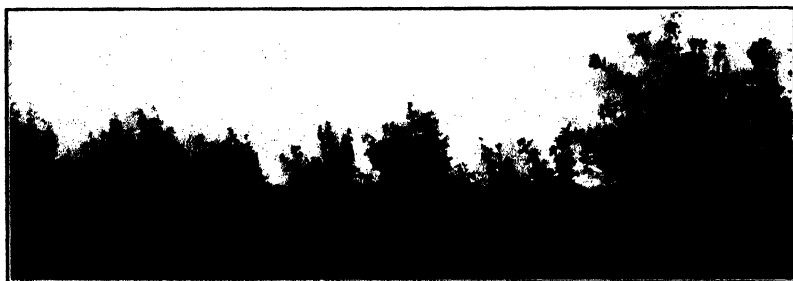
FIGURE 3.—Healthy control alfalfa plants at extreme right. Other plants show typical dwarf resulting from grafting by method illustrated in figure 2. One-hundred-percent infection was obtained in this experiment. Grafted June 7, 1934, and photographed September 9, 1934.

DISCUSSION

The presence of bacterialike bodies in the ducts of the roots, crowns, and bases of the stems of dwarfed alfalfa plants, even in very early stages of the disease, at first created the impression that they must be the causal agents. Isolations showed that not one but many different bacteria were present in the tissue, none of which would reproduce the disease under the experimental conditions provided.

The top symptoms of alfalfa plants affected with dwarf, such as the darker green foliage, are not unlike those of other plants affected with certain virus diseases; for example, phony disease of peach (*5*), spindle tuber and curly dwarf of potato (*4*), dwarf disease of rice (*3*), and a dwarf disease of sugarcane (*2*).

The presence of so much yellowing in the wood of the root and the plugging of the vessels with gum in which the bacterialike bodies are embedded constitute the chief differences between the dwarf disease of alfalfa and most other virus diseases. The presence of gum in the xylem vessels of plants affected with virus diseases is not unknown. Xylem vessels of potato plants affected with top necrosis may become filled with gum (*1*). Some plugging of vessels in the roots of lettuce (*Lactuca sativa* L.) plants affected with spotted wilt have been observed. Likewise a brown discoloration in the vascular system of lupine has been described (*7*).

Not only is the plugging of the xylem vessels with gum known to occur in the case of some virus diseases, but bacteria also have been found closely associated with some virus-affected plants *(6, 7, 8, 9)*.

Clearly, then, the presence of gum in the xylem ducts and the association of bacteria and bacterialike bodies with the diseased tissue are not sufficient to exclude alfalfa dwarf from the group of virus diseases as at present constituted.

SUMMARY AND CONCLUSIONS

The data presented show that alfalfa dwarf is not due to an unfavorable soil condition and is not caused by a fungus. The possibility that dwarf is due to a bacterium may not be entirely excluded; however, all of the evidence obtained argues against such a conclusion. Bacteria are present in considerable abundance in at least 75 percent of the infected roots, but none of those isolated caused infection under the conditions of the experiments.

The causal agent of dwarf does not live in soil free of diseased plants. Pieces of diseased roots added to the soil appeared to serve as a source of inoculum in some experiments, but stems from diseased plants did not.

Dwarf spreads readily from diseased to nearby healthy plants.

Water in itself is not an important factor in disseminating dwarf, although it might wash out and transport diseased living root tissue or possibly carry an insect vector.

Cuttings of the stems of diseased plants contained the causal agent and produced from 35 to 80 percent of diseased plants.

Leaf mutilation and pinprick methods of inoculation gave negative results. Likewise, juice inoculations failed to give infection.

Inserting bits of tissue from diseased plants into wounds in healthy plants failed to produce infection except when yellow wood of the root or crown was used. Even then the percentage of infection was very small except in one experiment in which 84-percent infection was obtained. Out of a total of 672 plants inoculated with yellow xylem 63, or 9 percent, became diseased.

Any method of grafting in which diseased and healthy root tissue formed a union gave a high percentage of transmission. One hundred-percent transmission was obtained in one experiment and 90 percent in another by grafting a piece of diseased root onto the lower end of the taproot of the healthy plant. This method gave the highest percentage of infection of any tried, presumably because a better union was formed.

The evidence presented seems to show that alfalfa dwarf is a virus disease belonging to the group that can be transmitted by grafting, but not by juice inoculation.

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NUTRITIVE VALUE OF THE PROTEIN IN CALF LUNGS, COW UDDERS, AND HOG SPLEENS[1]

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INTRODUCTION

In the commercial slaughter of meat food animals in the United States, the various meat byproducts are utilized in the manufacture of meat food products and animal feed. The nutritive value of meat byproducts is naturally of considerable interest both to the meat packer and to the consumer. In previous papers the writers have reported the nutritive value of the protein in a number of meat byproducts. The present paper gives similar information for calf lungs, cow udders, and hog spleens, together with some data on cracklings. These products, although used only to a limited extent for human food, are extensively employed in the manufacture of animal feed. The purpose of the experiments here reported was to determine the nutritive value of the protein in each meat byproduct as compared with that of the protein in lean beef, and the value of the proteins in the several animal tissues as supplements for each other and for the protein in corn meal.

PREVIOUS INVESTIGATIONS

The protein in beef spleen was found by Hoagland and Snider [2] to have practically the same value for maintenance and growth in young rats as the protein in veal, ox and hog brains, ox and hog tongues, and dried milk. In a later paper these authors [3] reported that the protein in calf lungs had an appreciably lower nutritive value than that in veal. Cracklings were found to contain protein of very low nutritive value, since 15 percent of protein from this source was required for maintenance in rats.

The protein in beef was found by Hoagland and Snider [4] to have a marked supplemental relationship for the protein in corn meal, wheat, bolted wheat flour, and oatmeal. When one part of beef protein was fed in a mixture with two parts of any one of the vegetable proteins, the mixture had the same value for maintenance and growth as a like quantity of beef protein alone.

Mitchell and Carman [5] found that the protein in both beef and veal supplemented the protein in patent white flour. When two parts of white-flour protein was fed in combination with one part

of beef or veal protein, the protein in the mixture had as high a value as that in beef alone.

EXPERIMENTAL PROCEDURE

Hog spleens, calf lungs, and beef round were purchased from local meat-packing establishments, and cow udders and cracklings were obtained from Chicago. Federal meat inspection was maintained at these establishments, and the products purchased had been inspected and passed for food purposes. Each meat byproduct was trimmed free of extraneous tissue, ground, and dried in an oven in a current of air at a temperature not exceeding 60° C. The dried products were ground fine and stored at approximately 2° C. until their nutritive value was tested by feeding to young, male, albino rats.

Each lot of the dried tissue was analyzed for nitrogen and fat. Each feed mixture was then made up to contain 8 percent of protein (N × 6.25) derived from the product or products to be tested. In addition, approximately 0.9 percent of protein was introduced in the 2 percent of yeast concentrate added as a source of vitamins B and G. One percent of cod-liver oil supplied vitamins A and D. The fat content of each mixture was adjusted to 4.4 percent by the addition of a sufficient quantity of lard. Four percent of ash and sufficient cassava starch to make 100 percent comprised the remainder of the mixture. When needed, each mixture was made up in the quantity of 1,000 g and was stored in a tightly covered glass jar for use during the experiment.

The rats used in these experiments were selected from a colony of albino rats which has been maintained for a number of years by the Bureau's biochemic laboratory. Litters of more than eight rats were reduced to that number within a few days after birth. Only male rats that weighed at least 40 g within 30 days after birth were used. Each group of rats fed a particular ration was selected from four or more litters.

Each ration was fed to a group of from five to eight rats for 60 days, and the experimental data are tabulated both for the first 30 days and for the entire period. Each rat was kept in a cylindrical wire cage with a raised screen bottom. The ration was supplied in a self-feeder. The rats were weighed twice weekly at regular intervals, and records were kept of the feed consumed.

RESULTS OF EXPERIMENTS

The results of the feeding experiments are presented in table 1. In judging the relative nutritive value of the protein in the different products, the average gain in weight per gram of protein consumed by each group of rats is of particular significance. Those experiments in which only one product was the main source of protein in the ration indicate that beef had the highest value, followed closely by calf lungs and hog spleens; cow udders and corn meal had much lower values. The nutritive value of cracklings as the main source of protein in the diet was not determined in these experiments since the writers [6] had previously found the protein in this product to be of very poor quality.

HOAGLAND, R., and SNIDER, G. G. See footnote 3.

TABLE 1.—*Comparative nutritive values of the proteins in various meats and meat byproducts fed singly, in combination among themselves, or with corn meal, to young male, albino rats*

Source of protein tested	Rat no.	Age of rats at beginning of test	Initial weight of rats	Gains and intake of rats in 30 days							Gains and intake of rats in 60 days						
				Total gain in weight	Total intake of—		Intake, per gram gain in weight, of—		Gain, in weight, per gram of—		Total gain in weight	Total intake of—		Intake, per gram gain in weight, of—		Gain, in weight, per gram of—	
					Feed	Protein	Feed	Protein	Feed	Protein		Feed	Protein	Feed	Protein	Feed	Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Calf lungs	1244A	23	42	81	315	25.2	3.9	0.31	0.26	3.21	149	660	52.8	4.4	0.35	0.23	2.82
	1244B	23	43	83	320	25.6	3.9	.31	.26	3.24	171	740	59.2	4.3	.35	.23	2.89
	1244C	23	43	94	344	27.5	3.7	.29	.27	3.42	183	792	63.4	4.3	.33	.23	2.89
	1244D	22	40	79	322	25.8	4.1	.33	.25	3.06	149	708	56.6	4.8	.38	.21	2.63
	1244E	22	43	80	344	27.5	4.3	.34	.23	2.91	156	738	60.6	4.9	.39	.21	2.57
	1244F	24	41	74	312	25.0	4.2	.34	.24	2.96	155	720	57.6	4.6	.37	.22	2.69
Average		23	42	82	326	26.1	4.0	.32	.25	3.13	161	730	58.4	4.6	.37	.22	2.75
Calf lungs and yellow corn meal [1]	1245A	26	40	76	313	25.0	4.1	.33	.24	3.04	153	731	58.5	4.8	.38	.21	2.62
	1245B	24	44	111	403	32.2	3.6	.29	.28	3.45	215	902	72.2	4.2	.34	.24	2.98
	1245C	24	40	89	337	27.0	3.8	.30	.26	3.30	190	796	63.7	4.2	.34	.24	2.95
	1245D	24	40	110	369	31.1	3.5	.28	.28	3.54	224	896	71.7	3.9	.31	.26	3.19
	1245E	23	43	112	398	31.8	3.5	.28	.28	3.52	223	914	73.1	4.1	.33	.24	3.05
Average		24	41	100	368	29.4	3.7	.30	.27	3.37	202	848	67.8	4.2	.34	.24	2.96
Hog spleens	1248A	22	45	68	263	21.0	3.9	.31	.26	3.24	108	543	43.4	5.0	.40	.20	2.49
	1248B	24	45	99	371	29.7	3.7	.30	.27	3.33	209	897	71.8	4.3	.34	.23	2.91
	1248C	25	42	69	290	23.2	4.2	.34	.24	2.97	122	626	50.1	5.1	.41	.19	2.44
	1248D	21	37	86	343	27.4	4.0	.32	.25	3.14	176	811	64.9	4.6	.37	.22	2.71
	1248E	21	44	76	352	26.6	4.4	.35	.23	2.86	148	753	60.2	5.1	.41	.20	2.46
	1248F	25	47	81	350	28.0	4.3	.35	.23	2.89	162	777	62.2	4.8	.38	.21	2.60
Average		23	43	80	325	26.0	4.1	.33	.25	3.07	154	735	58.8	4.8	.39	.21	2.60
Hog spleens and yellow corn meal [1]	1249A	28	44	108	381	30.5	3.5	.28	.28	3.54	163	773	61.8	4.7	.38	.21	2.64
	1249B	21	40	119	421	33.7	3.5	.28	.28	3.53	224	952	76.2	4.3	.34	.24	2.94
	1249C	21	40	94	355	28.4	3.8	.30	.26	3.31	196	844	67.5	4.3	.34	.23	2.90
	1249D	25	46	132	448	35.8	3.4	.27	.29	3.69	264	1,009	80.7	3.8	.31	.26	3.27
	1249E	24	44	134	456	36.5	3.4	.27	.29	3.67	234	1,067	85.4	4.6	.36	.22	3.74
	1249F	24	43	114	428	34.2	3.8	.30	.27	3.33	211	937	75.0	4.4	.36	.23	2.81
Average		24	43	117	415	33.2	3.6	.28	.28	3.51	215	930	74.4	4.4	.35	.23	2.88

[1] 4 percent of protein from each product.

TABLE 1.—*Comparative nutritive values of the proteins in various meats and meal byproducts fed singly, in combination among themselves or with corn meal, to young male, albino rats*—Continued

Source of protein tested	Rat no.	Age of rats at beginning of test	Initial weight of rats	Gains and intake of rats in 30 days							Gains and intake of rats in 60 days						
				Total gain in weight	Total intake of— Feed	Total intake of— Protein	Intake, per gram gain in weight, of— Feed	Intake, per gram gain in weight, of— Protein	Gain, in weight, per gram of— Feed	Gain, in weight, per gram of— Protein	Total gain in weight	Total intake of— Feed	Total intake of— Protein	Intake, per gram gain in weight, of— Feed	Intake, per gram gain in weight, of— Protein	Gain, in weight, per gram of— Feed	Gain, in weight, per gram of— Protein
		Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Cow udders	1268A	24	46	70	313	25.0	4.5	.36	.22	2.80	116	671	53.7	5.8	.46	.17	2.16
	1268B	19	44	81	336	26.9	4.1	.33	.24	3.01	152	784	62.7	5.2	.41	.17	2.42
	1268C	20	40	62	295	23.6	4.8	.38	.21	2.63	129	720	57.6	5.6	.45	.18	2.24
	1268D	22	40	62	296	23.7	4.8	.38	.21	2.62	116	663	53.0	5.7	.46	.17	2.19
	1268E	23	47	45	263	21.0	5.8	.47	.17	2.14	90	586	46.9	6.5	.52	.15	1.92
	1268F	23	39	67	283	22.6	4.2	.34	.24	2.96	119	657	52.6	5.5	.44	.18	2.26
	1268G	27	40	49	217	17.4	4.4	.36	.23	2.82	90	499	39.9	5.5	.44	.18	2.26
	1268H	20	41	39	208	16.6	5.3	.43	.19	2.35	78	452	36.2	5.8	.46	.17	2.15
Average		22	42	59	276	22.1	4.7	.38	.21	2.67	111	629	50.3	5.7	.46	.17	2.20
Cow udders and yellow corn meal [1]	1269A	25	43	51	249	19.9	4.9	.39	.20	2.56	92	553	44.2	6.0	.48	.17	2.08
	1269B	20	42	53	263	21.0	5.0	.40	.20	2.52	126	638	51.0	5.1	.40	.20	2.47
	1269C	21	43	88	305	24.4	5.0	.36	.22	2.79	166	775	62.0	4.7	.37	.21	2.68
	1269D	23	40	56	283	22.6	5.1	.40	.20	2.48	112	644	51.5	5.8	.46	.17	2.17
	1269E	25	46	50	275	22.0	5.5	.44	.19	2.27	116	647	51.8	5.6	.45	.18	2.24
	1269F	20	41	50	222	17.8	4.4	.36	.23	2.81	106	503	40.2	4.7	.38	.21	2.64
	1269G	21	42	53	262	21.0	4.9	.40	.20	2.52	107	570	45.6	5.3	.43	.19	2.35
	1269H	22	42	77	345	27.6	4.5	.36	.22	2.79	156	747	59.8	4.8	.38	.21	2.61
Average		22	42	57	276	22.0	4.9	.39	.21	2.59	123	635	50.8	5.3	.42	.19	2.41
Hog spleens, calf lungs, cow udders, and cracklings [2]	1270A	20	42	51	208	16.6	4.1	.33	.25	3.07	88	449	35.9	5.1	.41	.20	2.45
	1270B	25	44	48	274	21.9	5.7	.46	.18	2.19	97	573	45.8	5.9	.47	.17	2.12
	1270C	21	42	66	301	24.1	4.6	.37	.22	2.74	127	664	53.1	5.2	.42	.19	2.39
	1270D	21	48	58	296	23.7	5.1	.40	.20	2.49	106	621	49.7	5.9	.47	.17	2.13
	1270E	25	46	68	331	26.5	4.9	.39	.20	2.57	136	696	55.7	5.1	.41	.20	2.44
	1270F	24	45	74	234	18.7	3.2	.25	.32	3.96	136	593	47.4	4.4	.35	.23	2.87
	1270G	31	43	42	216	17.3	5.1	.41	.19	2.43	73	463	37.0	6.3	.51	.16	1.97
	1270H	22	44	44	182	14.6	4.1	.33	.24	3.01	82	407	32.6	5.0	.40	.20	2.52
Average		24	44	57	255	20.4	4.6	.37	.23	2.81	106	558	44.7	5.4	.43	.19	2.36

Sample																
Hog spleens, calf lungs, cow udders, cracklings, and yellow corn meal [3]																
1271A	28	42	44	180	14.4	5.5	44	.18	2.29	79	420	33.6	5.3	.43	.19	2.35
1271B	22	43	33	176	14.1	4.2	34	.24	2.98	88	393	31.4	4.5	.36		2.80
1271C	22	43	42	235	18.8	3.6	45	.18	2.23	85	486	38.9	5.7	.46	.17	2.18
1271D	26	39	40	225	18.0	5.6	45	.18	2.22	77	468	37.4	5.6	.49	.16	2.06
1271E	22	39	36	154	12.3	4.3	34	.23	2.93	84	366	29.3	4.4	.35	.23	2.87
1271F	23	41	43	182	14.6	4.2	34	.24	2.95	91	412	33.0	4.5	.36	.22	2.76
1271G	25	42	47	209	16.7	4.4	36	.22	2.81	114	488	39.0	4.3	.34	.81	2.92
1271H	25	47	36	169	13.5	4.7	38	.21	2.67	89	406	32.5	4.6	.37	.22	2.74
Average	25	42	40	191	15.3	4.8	39	.21	2.64	88	430	34.4	4.9	.40	.21	2.59
Beef round																
1256A	22	46	90	366	29.3	4.1	33	.25	3.07	190	824	65.9	4.3	.35	.23	2.88
1256B	22	45	95	358	28.6	3.8	30	.27	3.32	209	951	76.1	4.6	.36	.24	2.75
1256C	22	40	90	328	26.2	3.6	29	.27	3.44	183	764	61.1	4.2	.33	.24	3.00
1256D	23	43	91	349	27.9	3.8	31	.26	3.26	178	796	63.7	4.5	.36	.21	2.79
1256E	25	41	72	294	23.5	4.1	33	.24	3.06	141	666	53.3	4.7	.38	.27	2.65
1256F	25	43	97	354	28.3	3.6	29	.27	3.43	177	668	53.4	4.8	.38	.22	3.31
1256G	25	40	92	344	27.5	3.7	30	.26	3.35	165	754	60.3	4.6	.37	.24	3.74
1256H	23	40	86	337	27.0	3.9	31	.24	3.19	152	705	56.4	4.6	.37	.25	2.70
Average	23	42	89	341	27.3	3.8	31	.26	3.27	174	766	61.3	4.4	.35	.23	2.85
Beef round and yellow corn meal [1]																
1257A	23	42	98	364	29.4	3.7	30	.27	3.37	191	821	65.7	4.3	.34	.23	2.91
1257B	24	40	100	368	29.4	3.7	29	.27	3.40	221	885	70.8	4.0	.32	.22	3.12
1257C	21	41	82	298	23.8	3.6	29	.28	3.45	182	735	58.8	4.0	.32	.24	3.10
1257D	21	44	109	388	31.0	3.6	25	.28	3.52	226	887	71.0	3.9	.31	.22	3.18
1257E	24	41	93	347	27.8	3.9	30	.27	3.35	185	789	63.1	4.3	.34	.23	2.93
1257F	25	40	84	326	26.1	3.9	31	.26	3.52	197	792	63.4	4.0	.32	.24	3.11
1257G	24	46	104	392	31.4	3.8	30	.27	3.31	226	927	74.2	4.1	.33	.22	3.05
1257H	21	39	79	325	26.0	4.1	33	.24	3.04	178	708	56.6	4.0	.32	.25	3.14
Average	23	42	94	351	28.1	3.8	30	.27	3.33	201	818	65.5	4.1	.33	.24	3.07
Yellow corn meal																
1243A	22	45	56	315	25.2	5.6	45	.18	2.22	121	712	57.0	5.9	.47	.17	2.12
1243B	22	45	60	322	25.8	5.4	43	.19	2.33	131	748	59.8	5.7	.46	.18	2.19
1243C	21	41	48	276	22.1	4.8	46	.17	2.17	104	635	50.8	6.1	.49	.16	2.05
1243D	23	41	55	290	23.2	5.3	42	.19	2.37	124	679	54.3	5.8	.44	.18	2.28
1243E	23	42	60	319	25.5	5.7	43	.19	2.35	126	731	58.5	5.8	.46	.18	2.15
1243F	19	41	51	289	23.1	5.7	45	.18	2.21	114	668	53.4	5.9	.47	.17	2.13
1250A	20	42	47	262	21.1	5.6	45	.18	2.24	72	550	44.0	7.6	.61	.13	1.64
1250B	21	40	47	264	21.1	5.6	45	.18	2.23	89	590	47.2	6.6	.53	.15	1.89
1250C	21	39	48	300	24.0	6.3	50	.16	2.00	91	623	49.8	6.8	.55	.14	1.83
1250D	21	40	39	263	21.0	6.7	54	.15	1.86	86	600	48.0	7.0	.56	.17	1.79
1250E	25	46	66	344	27.5	5.2	42	.19	2.40	128	741	59.3	5.8	.46	.17	2.16
1250F	24	46	65	350	28.0	5.4	43	.19	2.32	133	780	62.4	5.9	.47	.17	2.13
Average	22	42	54	300	24.0	5.7	45	.18	2.23	110	671	53.7	6.2	.50	.16	2.03

[1] 1 percent of protein from each product.   [2] 2 percent of protein from each product.   [3] 1 percent of protein from first 4 products; 4 percent from yellow corn meal.   [4] percent of protein from each product.

The experiments in which the rations contained mixtures of animal products and corn meal are of particular interest because in several of them the results indicate a marked supplementary relationship between the two kinds of protein. Two proteins may be said to supplement each other if a mixture of the two has a higher biological value than their calculated combined value based on the values determined for the proteins individually and the proportions in which they were combined. Obviously the proteins supplement each other if the mixture has as high or higher nutritive value than the better of the two proteins. In making such comparisons all proteins must have been fed at the same level of intake.

In calculating the probable biological value of the protein in a mixture of an animal product and corn meal, assuming that no supplementary relationship exists, the writers have used essentially the method followed by Mitchell and Carman.[7] For example, table 1 shows that the feeding of calf lungs resulted in an average gain in weight in 60 days of 2.75 g for each gram of protein consumed, corn meal a gain of 2.03 g, and a mixture of equal parts of lung and corn protein a gain of 2.96 g. If the two kinds of protein did not supplement each other, then the calculated value for the mixture would be $\frac{2.75+2.03}{2} = 2.39$ g. However, since the mixture of lung and corn proteins actually brought about a gain of 2.96 g for each gram of protein consumed, or an increase of 0.57 g over the calculated value, there was evidently a material supplementary relationship between the two kinds of protein. Furthermore, the animals made materially higher gains when fed the mixture of proteins, both for the 30- and the 60-day tests, than when fed lung protein alone.

The protein in hog spleens supplemented that in corn meal to a considerable degree since the mixture of the two products induced a materially larger gain in weight per gram of protein consumed, both for the 30- and the 60-day tests, than did hog spleens alone. Likewise, the values of 3.51 g and 2.88 g obtained from the mixture for the 30- and 60-day tests, respectively, were materially higher than the calculated values of 2.65 g and 2.32 g.

Beef protein also supplemented that in corn meal materially since the mixture induced a larger gain in weight per gram of protein consumed than did beef alone. Furthermore, the actual gains in weight per gram of protein consumed in the mixture, both for the 30- and the 60-day tests (3.33 g and 3.07 g), were materially greater than the calculated values of 2.75 g and 2.44 g.

The experiments with cow udders and corn meal show that the udders alone induced a slightly greater gain in weight per gram of protein consumed during the 30-day test and a somewhat smaller gain during the 60-day test than the mixture of udders and corn meal. These results alone fail to disclose any supplementary relationship between the two kinds of protein in the 30-day test, and suggest only a slight supplementary value for the 60-day test. However, if the values determined through the experiment for the mixtures are compared with the calculated values, it appears probable that the two kinds of protein supplemented each other to a slight extent in the

[7] MITCHELL, H. H., and CARMAN, G. G. See footnote 5.

30- and the 60-day tests. For the 30-day test the calculated value was 2.45 g as compared with the determined value of 2.59 g, with a difference of +0.14 g, and for the 60-day test the values were 2.12 g and 2.41 g, respectively, with a difference of +0.29 g.

The experiment with a mixture containing 2 percent each of protein from hog spleens, calf lungs, cow udders, and cracklings failed to disclose any supplementary relationship among the several kinds of protein. The nutritive value of the mixed proteins was similar to that of cow udders alone.

The experiment with a mixture containing 1 percent each of protein from hog spleens, calf lungs, cow udders, and cracklings, and 4 percent of corn protein indicated a slight supplementary relationship between the animal and the corn proteins. The calculated gain in weight per gram of protein consumed in 30 days was 2.52 g, as compared with the experimental value of 2.64 g, with a difference of +0.12 g. The calculated value for the 60-day test was 2.20 g, as compared with the experimental value of 2.59 g, with a difference of +0.39 g.

SUMMARY

In this paper are reported the results of feeding experiments with young male albino rats to determine (1) the nutritive value, for maintenance and growth, of the protein in calf lungs, cow udders, and hog spleens as compared with that in beef; (2) the supplemental value of the proteins in the several animal products for one another; (3) the supplemental value of the protein in each animal product for that in corn meal; and (4) the supplemental value of a mixture of the proteins in several animal products for the protein in corn meal. The results of the experiments indicate the following:

(1) Beef had the highest value as a source of protein for maintenance and growth, followed closely by calf lungs and hog spleens, whereas cow udders had much the lowest value.

(2) The proteins in calf lungs, hog spleens, cow udders, and cracklings did not seem to supplement one another materially.

(3) The protein in calf lungs, hog spleens, and beef each supplemented the protein in corn meal to a considerable degree. The protein in cow udders appeared to supplement corn protein to a slight extent.

(4) The proteins in a mixture of hog spleens, calf lungs, cow udders, cracklings, and corn meal appeared to supplement one another to a slight degree.

EFFECT OF DIET, RANGE, AND FATTENING ON THE PHYSICAL AND CHEMICAL COMPOSITION OF COCKERELS [1]

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INTRODUCTION

A large proportion of the chickens sold for food in the United States are prepared for market in commercial feeding stations. The birds are confined in batteries and are fed heavily for various lengths of time on a wet mash which usually contains milk in some form. One major object of this procedure is to improve the quality of the carcasses of the birds.

The composition of the finished product of the poultry-fattening plants is one measure of the quality of the product, and may be affected by a number of factors. Of these the fattening process itself, carefully controlled in the feeding stations, is naturally of considerable importance. Other factors, not so easily controlled, are the diet of the birds previous to fattening and the degree of confinement during the rearing period. The latter is particularly important because of the increasing tendency of producers to rear their poultry in confinement. A study of the effect of all these factors on the composition of chickens should be of value, not only to those who fatten poultry for market, but to those who produce the birds as well.

PREVIOUS INVESTIGATIONS

Investigations of the effect of fattening on the chemical composition of poultry have been reported by Pfeiffer (9) [2] and Köhler (5). These investigations, however, were carried out more than 30 years ago, were made on a very limited number of birds, and did not involve an analysis of the various edible parts.

A few more recent contributions, which give the composition of chickens before and after fattening, are to be found in the literature, but the various conditions during the rearing period apparently have not been studied in this way. Lee (6) and Mairs (7) have reported the percentage which the dressed weight is of the live weight, the former investigator in fattened birds and the latter in unfattened birds. Jull and Maw (4) and Maw (8) have reported the percentage of dressed weight and of the edible portions in broilers and roasting chickens before and after fattening. Hepburn and Holder (3) have reported the results of physical and chemical analyses of fattened and unfattened birds.

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The present paper reports a study of the effects of the diet fed during the rearing period, of range as compared with confinement, and of fattening on the physical and chemical composition of the edible portions of cockerels.

METHODS OF INVESTIGATION

Several groups of chickens were reared on various diets during 1927, 1928, and 1929. All of the birds were reared on range in 1927, but in 1928 and 1929 some of the birds on each diet were confined and some were allowed on range. An average of about 15 cockerels were reared in each group the first year, 32 the second year, and 60 the third.

Barred Plymouth Rock chicks were obtained from a commercial hatchery for the experiments of the first 2 years. During the third year's experiment, Rhode Island Red chicks, hatched from eggs produced by the breeding flock of the United States Animal Husbandry Experiment Farm, Beltsville, Md., were used.

In 1927, the chicks were hatched on April 18 and were put on feed 2 days later. The chicks raised during the second year were hatched on April 21 and were fed for the first time on April 24. In 1929, the chicks were hatched from two settings, one hatch occurring on April 8 and the other April 10, and were fed first on the day after hatching. All the chicks were hatched in incubators and brooded under similar conditions. They were kept in the brooder houses the first 2 days and allowed outdoors thereafter when the weather permitted. The range birds were brooded in colony brooder houses 10 feet square and were given access to a grass range 100 feet square. The confined birds were brooded in 8- by 15-foot rooms in a permanent brooder house and were allowed outdoors in 8- by 12-foot concrete-floored run yards.

The percentages of the ingredients used in the different diets are given in table 1. Separate groups of birds were reared on diets 0, 1, 2, 3, 4, 5, 7, and 27 in 1927; on diets 1, 2, 5, 6, 9, 10, and 29 in 1928; and on diets 1, 29, and 34 in 1929.

TABLE 1.—*Composition of the rearing diets used in experiments, 1927–29*

Diet no.	Corn, ground, yellow	Buttermilk, dried	Sodium chloride	Calcium carbonate	Bonemeal, steamed	Meat scrap	Meat meal, 72 percent protein	Fish meal	Pig liver, dried	Cod-liver oil	Menhaden fish oil	Alfalfa - leaf meal	Corn gluten meal	Bran, wheat	Middlings, wheat	Oats, rolled
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
0	40.0	4.0	1.0	2.0	3.0		8.0					2.0		10.0	10.0	20.0
1	62.5	37.5														
2	62.2	37.3								0.5						
3	61.9	37.1									1.0					
4	60.6	36.4	1.0	2.0												
5	60.3	36.2	1.0	2.0							.5					
6	60.0	36.0	1.0	2.0					1.0							
7	50.6	36.4	1.0	2.0										10.0		
9	60.6	31.4	1.0	2.0	5.0											
10	60.6	31.4	1.0	2.0				5.0								
27	50.6	36.4	1.0	2.0								10.0				
29	60.6	26.4	1.0	2.0									10.0			
34	50.6	16.4	1.0	2.0									20.0	10.0		

Diet 0 has given good results in rearing chickens at the experiment farm and is included for comparative purposes. Diet 1, the basal diet, was composed entirely of ground yellow corn and dried buttermilk mixed in such proportions as to contain approximately 18 percent of protein. The remaining diets contained various supplements to diet 1.

When the birds were nearly mature, the pullets were separated from the males and two representative cockerels from each group were killed and analyzed. At the same time, six other males from each group were placed in fattening batteries. The ages of these cockerels were 23 weeks in 1927, 25 weeks in 1928, and 20 weeks in 1929. The feed mixtures used for the fattening period in the respective years are shown in table 2. Twice each day the birds were allowed all they would consume of these wet mashes. After a 14-day fattening period, two representatives of the fattened birds from each group were killed and analyzed.

TABLE 2.—*Composition of the fattening mashes used in experiments, 1927–29*

Year	Corn, ground, yellow	Oats, rolled	Middlings, wheat	Flour, red-dog	Skim milk, dried	Butter-milk, dried	Water added
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1927	24	16				6	54
1928	24		3	12		7	54
1929	24	16				6	54

The birds were weighed just before they were killed. They were then bled, picked, and weighed again to obtain the dressed weights. Each of the carcasses was separated to obtain samples of breast muscle, leg muscle, and remaining edible portion. The last-mentioned sample was composed of all the edible material (including the heart, liver, and empty gizzard) except the breast and leg muscles. All the surface fat was removed from the breast and leg and was placed in the remaining edible sample, so that only that fatty tissue which was between or within the individual muscles of the breast and leg remained with these samples. The weights of the three samples were recorded.

The protein, fat, ash, and water of the breast muscle, the leg muscle, and the remaining edible portion were determined, and, from the results of these analyses, the composition of the total edible portion was calculated. The chemical determinations were made according to the methods of the Association of Official Agricultural Chemists (*1*): The water by distillation with toluene, the fat by extraction with ether, the ash by ignition to a red heat, and the nitrogen by the Kjeldahl method. The protein was calculated from the total nitrogen by use of the factor 6.25.

The results of the physical and chemical analyses of the individual cockerels were studied statistically by Fisher's (*2*) method of variance analysis.

RESULTS OF THE INVESTIGATION

PHYSICAL COMPOSITION

The results of the physical analyses of the birds are presented in tables 3, 4, and 5. The data on individual birds were too numerous to warrant their presentation in full, but the averages obtained under the different conditions each year and for the 3 years combined are presented in these tables.

TABLE 3.—*Ratios of the various edible portions of the cockerels, reared on the different diets, to their dressed weights*

Diet	Year	Birds	Breast muscle	Leg muscle	Remaining edible portion	Total edible portion
		Number	*Percent*	*Percent*	*Percent*	*Percent*
0	1927	4	13. 1	21. 1	21. 8	56. 0
1	1927	4	12. 7	19. 3	24. 8	56. 8
	1928	8	13. 2	19. 3	22. 4	54. 7
	1929	8	13. 0	18. 7	17. 6	49. 3
	Total or average	20	13. 0	19. 1	21. 0	52. 9
2	1927	4	12. 3	19. 8	20. 6	52. 7
	1928	8	13. 5	19. 7	22. 0	55. 3
	Total or average	12	13. 1	19. 8	21. 6	54. 4
3	1927	4	13. 6	19. 8	21. 8	55. 2
4	1927	4	12. 3	19. 9	23. 8	56. 0
5	1927	4	13. 2	19. 9	23. 6	56. 7
	1928	8	11. 7	19. 1	22. 4	53. 3
	Total or average	12	12. 2	19. 4	22. 8	54. 4
6	1928	8	13. 3	19. 7	21. 9	54. 9
7	1927	4	13. 2	20. 6	20. 8	54. 6
9	1928	8	12. 1	19. 6	23. 0	54. 7
10	1928	8	13. 0	19. 8	20. 5	53. 4
27	1927	4	12. 0	19. 7	22. 9	54. 6
29	1928	8	13. 0	20. 5	22. 7	56. 1
	1929	8	12. 9	18. 7	17. 3	48. 9
	Total or average	16	13. 0	19. 6	20. 0	52. 5
34	1929	8	12. 9	19. 4	18. 0	50. 4

TABLE 4.—*Ratios of the various edible portions of the cockerels, reared in confinement and on range, to their dressed weights*

Year	Birds	Breast muscle			Leg muscle			Remaining edible portion			Total edible portion		
		Confined birds [1]	Range birds	Difference [2]	Confined birds [1]	Range birds	Difference [2]	Confined birds [1]	Range birds	Difference [2]	Confined birds [1]	Range birds	Difference [2]
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1927	32		12. 80			20. 01			22. 52			55. 34	
1928	28	12. 36	13. 32	7. 8	18. 73	20. 63	10. 1	21. 45	22. 83	6. 4	52. 55	56. 69	7. 9
1929	12	12. 85	13. 01	1. 2	18. 07	19. 80	9. 6	17. 23	18. 05	4. 8	48. 15	50. 86	5. 6
Total or average	72	12. 51	13. 04	4. 2	18. 53	20. 22	9. 1	20. 18	21. 90	11. 1	51. 23	55. 12	7. 6

[1] No birds were reared in confinement in 1927.
[2] Percentage increase in weight of birds reared on range.

TABLE 5.—*Ratios of the various edible portions of the cockerels, some before and others after fattening, to their dressed weights*

Year	Birds	Breast muscle			Leg muscle			Remaining edible portion			Total edible portion		
		Before fattening	After fattening	Difference [1]	Before fattening	After fattening	Difference [1]	Before fattening	After fattening	Difference [1]	Before fattening	After fattening	Difference [1]
	No.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
1927	16	12.97	12.63	−2.6	20.12	19.89	−1.1	20.02	25.02	+25.0	53.15	57.53	+8.2
1928	28	13.14	12.70	−3.3	19.02	20.02	+5.3	21.24	23.24	+9.4	53.96	55.41	+2.7
1929	12	12.94	12.95	+1.1	18.48	19.50	+5.5	15.95	19.41	+21.7	47.36	51.81	+9.4
Total or average	56	13.05	12.73	−2.5	19.49	19.87	+1.9	19.75	22.97	+16.3	52.30	55.28	+5.7

[1] Percentage gain or loss during fattening period.

The results of the statistical analysis brought out the fact that on the whole the diets on which the birds were reared in this experiment had little effect on their physical composition. However, table 3 shows that the proportion of the breast muscle in the birds reared on diet 5 in 1928 was 10 percent less, on an average, than that of the birds reared on diets 1, 2, 6, 10, and 29. This difference was significant, the odds being greater than 19 to 1 that it was not due to chance.

The proportion of the edible portions in the carcasses of the birds reared on range was consistently higher than that of the birds reared in confinement. In the percentage which the breast muscle is of the dressed weight, the range birds were about 8 percent higher in 1928 than the confined birds. The proportion of leg muscle was about 10 percent greater in the range birds than in the confined birds in both 1928 and 1929. These differences were highly significant, the odds being more than 99 to 1 that they were not due to chance. Likewise, the proportion of total edible portion was 7.9 percent greater in 1928 and 5.6 percent greater in 1929 in the range birds than in the confined. The odds were greater than 99 to 1 in 1928 and 19 to 1 in 1929 that these differences in the total edible portions were not due to chance. The percentage of remaining edible portion was also higher in the range birds, but the difference was not consistent enough to be statistically significant.

Between the fattened and unfattened birds, little difference was found in the percentages of breast muscle and leg muscle and these differences were not significant. However, there was an increase in the weight of these edible portions during the 2-week fattening period. This increase in weight was 14 percent in the case of the breast muscle and 19 percent in that of the leg muscle, on an average, for the 3 years. The greatest and most consistent differences in the results of the physical analyses were found in the remaining edible portion of the carcasses analyzed before and after fattening. The carcasses of the fattened birds also contained a greater percentage of total edible portion than did those of the unfattened, the differences ranging from about 3 to 9 percent for the different years.

CHEMICAL COMPOSITION

The average chemical composition of the edible portions of the cockerels reared under the different conditions of diet, range, and fattening is shown in tables 6, 7, and 8.

TABLE 6.—*Average chemical composition of the edible portions of the cockerels reared on the different diets*

Diet no.	Year	Birds	Breast muscle				Leg muscle			
			Protein	Fat	Ash	Water	Protein	Fat	Ash	Water
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	1927	4	24.3	0.79	1.11	73.5	21.0	3.74	1.12	73.5
1	1927	4	24.3	.87	1.11	73.3	20.9	4.80	1.04	73.0
	1928	8	24.2	.91	1.11	74.3	20.6	4.70	1.08	73.9
	1929	8	24.5	.51	1.05	73.7	21.0	3.87	1.02	74.1
	Total or average	20	24.3	.74	1.09	73.9	20.8	4.39	1.05	73.8
2	1927	4	24.1	.65	1.14	73.9	20.8	4.86	1.04	73.1
	1928	8	24.3	.84	1.09	74.5	20.7	4.79	1.05	74.1
	Total or average	12	24.2	.78	1.11	74.3	20.7	4.81	1.05	73.7
3	1927	4	24.4	.88	1.22	73.6	20.8	4.98	1.09	72.9
4	1927	4	24.8	.82	1.14	73.3	21.3	4.48	1.12	73.0
5	1927	4	24.5	.65	1.24	73.5	21.1	4.42	1.05	72.8
	1928	8	23.8	.78	1.07	74.7	20.6	4.40	.99	74.1
	Total or average	12	24.0	.74	1.13	74.3	20.8	4.41	1.01	73.7
6	1928	8	24.3	.78	1.11	74.3	20.7	4.25	1.00	74.2
7	1927	4	24.6	1.03	1.14	73.7	21.1	4.70	1.12	73.0
9	1928	8	23.9	1.03	1.03	74.3	20.1	5.46	.97	73.6
10	1928	8	23.9	.84	1.05	74.3	20.1	4.58	1.02	74.6
27	1927	4	24.5	.98	1.13	73.6	20.7	4.93	1.09	73.3
29	1928	8	24.3	.99	1.06	74.1	20.7	4.47	.98	74.2
	1929	8	24.5	.45	1.07	73.8	21.3	3.73	1.02	74.0
	Total or average	16	24.4	.72	1.06	74.0	21.0	4.10	1.00	74.1
34	1929	8	24.6	.55	1.07	73.8	21.1	4.06	1.02	74.1

Diet no.	Year	Birds	Remaining edible portion				Total edible portion			
			Protein	Fat	Ash	Water	Protein	Fat	Ash	Water
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	1927	4	17.2	30.7	0.75	51.4	20.3	13.7	0.97	64.7
1	1927	4	15.6	36.4	.70	47.3	19.2	18.1	.90	61.6
	1928	8	17.8	25.1	.80	56.3	20.3	12.4	.97	66.6
	1929	8	19.2	21.9	.82	59.3	21.3	9.5	.96	68.2
	Total or average	20	17.9	26.1	.79	55.7	20.5	12.4	.95	66.2
2	1927	4	17.0	28.6	.77	53.7	20.0	13.2	.95	65.6
	1928	8	16.8	30.5	.69	52.2	20.0	14.1	.91	65.4
	Total or average	12	16.9	29.9	.72	52.7	20.0	13.8	.92	65.5
3	1927	4	16.3	32.0	.72	50.4	19.9	14.9	.97	64.0
4	1927	4	15.9	34.4	.71	48.4	19.7	16.7	.95	62.6
5	1927	4	15.9	34.7	.68	48.5	19.7	16.2	.95	62.9
	1928	8	17.5	26.8	.76	54.9	19.9	13.4	.91	63.9
	Total or average	12	17.0	29.4	.73	52.7	19.8	14.3	.92	63.5
6	1928	8	18.3	24.0	.80	57.2	20.6	11.4	.95	67.3
7	1927	4	17.5	27.8	.74	54.1	20.5	12.8	.98	65.9
9	1928	8	16.8	30.5	.76	51.8	19.5	15.3	.89	63.6
10	1928	8	18.6	22.2	.85	58.4	20.3	10.8	.96	68.0
27	1927	4	16.0	33.7	.67	49.7	19.4	16.4	.93	63.1
29	1928	8	17.5	29.0	.79	53.0	20.2	13.6	.92	65.6
	1929	8	19.7	20.8	.78	59.8	21.6	9.0	.95	68.8
	Total or average	16	18.6	24.9	.78	56.4	20.9	11.3	.93	67.2
34	1929	8	19.0	22.6	.83	58.4	21.2	9.9	.96	68.3

TABLE 7.—*Average chemical composition of the edible portions of the cockerels reared in confinement and on range*

BREAST MUSCLE

Year	Birds	Protein content of—		Fat content of—		Ash content of—		Water content of—	
		Con-fined [1] birds	Range birds	Con-fined [1] birds	Range birds	Con-fined [1] birds	Range birds	Con-fined [1] birds	Range birds
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1927	32		24.4		0.83		1.15		73.5
1928	28	23.7	24.4	0.85	.92	1.06	1.08	74.6	74.1
1929	12	24.6	24.5	.47	.53	1.07	1.07	73.6	74.0
Total or average	72	24.0	24.4	.71	.81	1.06	1.11	74.3	73.8

LEG MUSCLE

Year	Birds	Con-fined birds	Range birds	Con-fined birds	Range birds	Con-fined birds	Range birds	Con-fined birds	Range birds
1927	32		21.0		4.55		1.08		73.1
1928	28	20.1	20.9	4.96	4.36	0.99	1.04	74.2	74.0
1929	12	21.2	21.1	3.62	4.15	1.02	1.02	74.3	73.9
Total or average	72	20.4	21.0	4.56	4.41	1.00	1.05	74.2	73.6

REMAINING EDIBLE PORTION

Year	Birds	Con-fined birds	Range birds	Con-fined birds	Range birds	Con-fined birds	Range birds	Con-fined birds	Range birds
1927	32		16.4		32.3		0.72		50.5
1928	28	17.8	17.4	24.9	28.9	0.80	.76	56.6	53.0
1929	12	19.5	19.1	21.1	22.4	.82	.80	50.9	58.4
Total or average	72	18.3	17.3	23.8	29.3	.81	.75	57.6	52.8

TOTAL EDIBLE PORTION

Year	Birds	Con-fined birds	Range birds	Con-fined birds	Range birds	Con-fined birds	Range birds	Con-fined birds	Range birds
1927	32		19.8		15.3		0.95		63.8
1928	28	19.9	20.3	12.4	13.6	0.93	.93	66.9	65.4
1929	12	21.4	21.2	9.1	9.8	.97	.95	68.7	68.0
Total or average	72	20.4	20.2	11.4	13.7	.94	.94	67.5	65.1

[1] No birds were raised in confinement in 1927.

There were practically no differences in the chemical composition of the edible portions of the cockerels which could be ascribed to diet. In three instances, once in breast muscle and twice in leg muscle, there was a difference of about 5 percent in the proportion of ash which could be attributed to diet, but the constituent of the diet responsible for this difference could not be determined.

The results of the chemical analyses reveal a few significant differences between the composition of the range birds and the confined birds. In 1928, in the leg muscle of the range birds the percentage of protein was almost 4 percent higher and that of the ash was more than 5 percent higher than in the confined birds. In the same year, the percentage of protein in the breast muscle was almost 3 percent greater in the range birds. These differences were all highly significant. The percentage of fat in the edible portions was generally higher in the range birds, but these differences were not statistically significant.

TABLE 8.—*Average chemical composition of the edible portions of the cockerels, some before and others after fattening*

BREAST MUSCLE

Year	Birds	Protein content		Fat content—		Ash content—		Water content—	
		Before fatten-ing	After fatten-ing	Before fatten-ing	After fatten-ing	Before fatten-ing	After fatten-ing	Before fatten-ing	After fatten-ing
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1927	16	24.4	24.5	0.68	0.98	1.16	1.14	74.0	73.1
1928	28	24.1	24.0	.58	1.19	1.10	1.05	74.6	74.1
1929	12	25.1	23.9	.33	.67	1.13	1.00	73.6	74.0
Total or average	56	24.4	24.1	.55	1.02	1.12	1.07	74.2	73.8

LEG MUSCLE

1927	16	21.4	20.6	3.62	5.48	1.10	1.07	73.3	72.9
1928	28	20.8	20.2	4.04	5.29	1.04	.99	74.4	73.8
1929	12	21.5	20.8	2.92	4.85	1.07	.97	74.5	73.7
Total or average	56	21.1	20.4	3.68	5.25	1.06	1.01	74.1	73.5

REMAINING EDIBLE PORTION

1927	16	18.3	14.6	24.8	39.8	0.80	0.64	56.1	44.9
1928	28	19.0	16.2	21.8	32.0	.84	.71	58.5	51.2
1929	12	21.2	17.4	15.7	27.8	.89	.73	63.6	54.6
Total or average	56	19.3	16.0	21.3	33.3	.84	.70	58.9	50.1

TOTAL EDIBLE PORTION

1927	16	20.9	18.8	10.9	19.6	1.00	0.90	66.9	60.7
1928	28	20.8	19.4	10.4	15.7	.97	.89	68.0	64.3
1929	12	22.4	20.3	6.5	12.4	1.03	.89	70.6	66.3
Total or average	56	21.2	19.4	9.7	16.1	.99	.89	68.3	63.7

Table 8 shows marked and consistent differences in composition between the fattened and unfattened birds. The percentage of fat was higher in all the edible portions of the fattened birds than it was in the same portions of the unfattened ones. The differences were about 85 percent in the breast muscle, 43 percent in the leg muscle, 57 percent in the remaining edible portion, and 66 percent in the total edible portion. However, in the breast muscle there was only 0.55 percent of fat on an average, before fattening, and the actual gain in the weight of the breast fat was, therefore, very small.

There was usually a lower percentage of protein, ash, and water in the edible portions of the fattened birds. These differences were uniformly significant in the remaining and total edible portions and, in most cases, in the breast and leg muscles. Attention is called to the fact that there was a gain in the weight of the protein, ash, and water, even though there was a decrease in the percentage of these constituents.

COMPOSITION OF THE GAIN IN WEIGHT DURING FATTENING

By means of the results obtained from the physical and chemical analyses of these cockerels, the composition of the gain in weight during the fattening period was calculated. A summary of the increase in the live weight, dressed weight, and in weight of the edible portions is given in table 9. The increase in the chemical constituents of the edible portions is presented in table 10.

TABLE 9.—*Average weights of the cockerels, some before and others after fattening, and of the edible portions of their carcasses, the gain in weight during fattening, and the relation of these weights and gains to the total live weight before fattening*

[Averages of 56 fattened and 56 unfattened cockerels]

Item	Before fattening		After fattening		Gain during fattening	
	Weight	Live weight before fattening	Weight	Live weight before fattening	Weight	Total live weight before fattening
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Live weight	2,203	100.00	2,530	114.84	327	14.84
Dressed weight [1]	1,878	85.25	2,196	99.68	318	■ 14.43
Breast muscle	245	11.12	280	12.71	35	1.59
Leg muscle	366	16.61	437	19.84	71	3.23
Remaining edible	371	16.84	505	22.92	134	6.08
Total edible	982	44.57	1,222	55.47	240	10.90

[1] The dressed weight is the weight of the carcass after killing and picking.

TABLE 10.—*Chemical distribution of the gain in weight of the edible portions of the cockerels during fattening*

[Average difference between 56 fattened and 56 unfattened cockerels]

Edible portion	Protein		Fat		Ash		Water		Total gain	
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Breast muscle	8	3.2	1.5	0.6	0.25	0.1	25	10.4	34.75	14.3
Leg muscle	12	5.0	9.0	3.7	.53	.2	50	20.8	71.53	29.7
Remaining edible portion	10	4.2	89.0	37.1	.42	.2	35	14.5	134.42	56.0
Total edible portion	30	12.4	99.5	41.4	1.20	.5	110	45.7	240.70	100.0

The increase in the total edible portions was about 73 percent, that of the remaining edible portion 41 percent, that of the leg muscle 21 percent, and that of the breast muscle 10 percent of the gain in live weight during fattening. In the edible portion alone, the gain in protein was 9 percent; in fat, 30 percent; in ash, about 0.4 percent; and in water, 33 percent of the gain in live weight.

These results show that the gain in weight of the birds consisted largely of an increase in fat and water in the parts included in the remaining edible portion, chiefly in fat deposits about the abdominal organs and under the skin. At the same time there was a noticeable gain in the other constituents and in the breast and leg muscle.

DISCUSSION

The fact that the birds reared on range had a higher percentage of breast muscle, leg muscle, and total edible portion than those reared in confinement is probably due, in part, to the greater opportunity for exercise afforded the range birds and, in part, to the feed which the birds were able to pick up on the range. Any factor which results in a higher proportion of breast or leg muscle merits practical consideration, for these are the most desirable portions from the consumer's standpoint, and they play a large part in determining the market grade of the finished carcass.

The results of this investigation have shown that, during the fattening period, relatively large quantities of fat and water were deposited in the adipose tissues of the bodies of the cockerels. At the same time there was an increase in the weight of water and fat in the breast and leg muscle and a small gain in the weight of protein and ash in all the edible tissues.

Since the birds used in these experiments were not mature when they were fattened, they would be expected to continue their growth during the fattening period. This continued growth accounts for the increase in the quantity of ash and protein and for at least part of the water. Such a gain is probably no greater than would have occurred on ordinary feeding for the same length of time. In young, rapidly growing birds, the increase in protein and ash would make up a greater proportion of the gains than in mature birds.

It has been frequently stated in the literature that a part of the water in the tissues of chickens is replaced by fat during the fattening process. Such a statement is probably based on the fact that the percentage of water in the tissues decreases with an increase in the percentage of fat. This decrease in the proportion of water, as well as that of the ash and protein, is a result of the diluting effect of the large deposition of fat in the tissues. As a matter of fact, there was an increase in the quantity of water, ash, and protein in all the edible portions. In the case of water, this increase in quantity was 10 percent greater than that of the fat.

Perhaps the picture may be presented more clearly if a calculation of the ratio of water to nitrogen in the edible portions is given. The results of this computation are presented in table 11, which shows that the ratio of water to nitrogen was slightly higher in every case after fattening than before and, in the gain during fattening, it was higher than either before or after fattening. Thus the proportion of water in the fat-free edible tissues tends to increase during fattening.

TABLE 11.—*Parts of water to 1 part of nitrogen in the edible portions of the cockerels, some before and others after fattening, and in the tissues constituting gain in weight during fattening*

Edible portion	Parts of water to 1 part of nitrogen in—		
	Edible portions before fattening	Edible portions after fattening	Gain during fattening
Breast muscle	19. 01	19. 11	19. 50
Leg muscle	21. 96	22. 47	26. 07
Remaining edible portion	19. 06	19. 63	21. 85
Total edible portion	20. 14	20. 54	23. 48

Table 11 also shows that the leg muscle contains a considerably greater proportion of water than the other edible portions. This difference was consistent in the fattened birds, in the unfattened birds, and in the composition of the gain during fattening.

SUMMARY

Several groups of chickens were reared each year for 3 years on a number of different diets, and, during the last 2 years, under opposite conditions of range and confinement. The diets fed were as follows: A normal diet which had given good results in rearing chickens and which was included for comparative purposes, a basal diet composed entirely of ground yellow corn and dried buttermilk, and a number of other diets which were formulated by substituting mineral supplements, fish oils, animal byproducts, or plant products in the basal diet.

When the birds were nearly mature, representative cockerels were selected from each group and analyzed. Other cockerels from each group were fattened for 2 weeks, and analyses were again made of representative birds.

For the purpose of making physical and chemical analyses, the edible portions of the carcasses were separated into 3 parts—the breast muscle, the leg muscle, and the remaining edible portion—and these parts were weighed. The percentages of protein, fat, ash, and water were then determined in each of these portions, and from the results the percentage composition of the total edible portion was calculated. The average results of these analyses are presented. The results were studied statistically by Fisher's method of variance analysis.

The study showed that the rearing diets used did not result in any significant differences in the physical composition of the birds in a great majority of cases.

The percentage of breast muscle was about 8 percent higher in 1928, that of leg muscle was about 10 percent higher in 1928 and 1929, and that of the total edible portion was about 8 percent higher in 1928 and 6 percent in 1929 in those birds which were reared on range than in those which were confined.

Fattening resulted in increases in the proportion of remaining edible portion (comprising all the edible portions except the breast and leg muscle), which ranged from nearly 10 percent in 1928 to 25 percent in 1927. In total edible portion there was an average increase of almost 6 percent for the 3 years due to fattening. No significant change in the percentage of breast or leg muscle took place during fattening, though there were gains of 14 percent in the weight of the breast muscle and 19 percent in that of the leg muscle.

The diets used had relatively little effect on the chemical composition of the edible portions. A few significant differences were found in the ash content of the breast and leg muscles of the birds reared on some of the diets, but the constituent responsible for these differences could not be determined.

In only 1 of the 2 years in which range was compared with confinement was there any significant chemical difference due to this factor. In 1928, the proportion of protein in the breast muscle was almost 3 percent greater, that of protein in the leg muscle was almost 4 per

cent greater, and that of ash in the leg muscle was 5 percent greater in the range birds than in the confined.

The birds which were fattened for 2 weeks had, on an average, 85 percent more fat in the breast muscle, 43 percent in the leg muscle, 57 percent in the remaining edible portion, and 66 percent in the total edible portion, than those which were not fattened.

The gain in live weight of the birds during fattening, as calculated from the analytical results, consisted of about 73 percent in the total edible portions, 41 percent in the remaining edible portions, 21 percent in the leg muscle, and 10 percent in the breast muscle. The gain in protein was 9 percent, that of the fat 30 percent, that of the ash 0.4 percent, and that of the water, 33 percent of the gain in live weight.

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THE RELATIVE VALUE OF VARIOUS LARDS AND OTHER FATS FOR THE DEEP-FAT FRYING OF POTATO CHIPS [1]

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INTRODUCTION

Notwithstanding the increase in the large-scale preparation of potato chips and other foods fried in deep fat there has been little scientific investigation published on the relative value of the various fats used for this purpose. To meet the demand for such information the Bureaus of Home Economics, Animal Industry, and Plant Industry during 1933 undertook cooperative experiments on nine fats used in the preparation of potato chips. Throughout these studies it was considered that a satisfactory fat for frying should be stable; should have a high smoking point and not deteriorate unduly during frying; and should produce a high-quality product that can be merchandised without too great a loss of quality.

REVIEW OF LITERATURE

A number of the studies made to determine the effects of various conditions of cookery upon fats have a bearing on this question of fats for deep frying. An early investigation by Fulmer and Manchester (5) [3] showed that the decrease in iodine value for cottonseed oil was hastened when the oil was heated above 180° C., but that temperatures below 220° produced little change in the free fatty acid content. Between 220° and 240° the acid percentage was more than doubled when the oil was heated for 10 minutes; after 30 minutes it was 4 times as great as in the original oil; and at 270° it was 9 and 15 times as great when the oil was heated for 10 and 30 minutes, respectively.

Upon comparing cottonseed oil and butter when fresh and when extracted from pastry baked at 200° C., Masters and Smith (9) found a decrease in iodine values and an increase in acetyl value, refractive index, and acidity in the heated fats. They concluded that the first change in heated fats might be hydroxylation of the unsaturated molecule. The oxidation was apparently measurable only when the fat was overcooked.

Blunt and Feeney (2) found that fat which has been used in cooking had both a lower smoking point and a higher acidity than did fresh fat.

Holmes and Lang (6) concluded that doughnuts fried in beef fat, lard, cottonseed oil, coconut fat, and peanut oil had the least fat

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absorption if the vegetable fats were heated 30° to 40° F. higher than the animal fats.

Cooking experiments conducted by Williams and Gray (*16*) led them to conclude that fat absorption was dependent more upon the kind of food and the temperature used than upon the kind of frying fat. They advocated the use of stable fats having little flavor, such as cottonseed products.

When Woodruff and Blunt (*17*) fried potato chips and doughs in lard and cottonseed oil they found that a longer period was required to secure the desired brown color of the product when lard was used. Potato chips fried in lard at 190° C. gave a fat absorption of 34.18 percent, while chips fried in cottonseed oil absorbed 34.4 percent. With doughnuts the amount of fat absorbed depended upon the temperature used and the period of frying, but not so with potato chips. The fat extracted from the foods fried in cottonseed oil showed less change in iodine number and acidity value than did that fried in lard. Woodruff and Blunt believed that the initial acidity of the lard accounted in part for its high acidity after heating. They found so little change in the oil that the effect of the time of frying was considered to be negligible. On the other hand, the fat absorbed by the fried food underwent greater chemical changes than the frying fat. They concluded that the appearance of a lard after frying gives no indication of its quality.

Morgan and Cozens (*10*) found that the fats used in frying doughs showed consistent decreases in iodine number and melting point but increases in acidity and refractive index after frying. They thought it possible that a relationship existed between the acidity of the fat and the quantity of fat absorbed by dough fried in lard, olive oil, and hydrogenated cottonseed oil at 210° C.

Sprague (*13*) stated that the reheating of a fat seemed to have little effect upon its quality if the temperature of the fat was kept low.

Vosbury (*15*), using leaf lard, a mixture of lard and suet, hydrogenated cottonseed oil, and cottonseed, coconut, and peanut oils, reported that a high-grade cottonseed oil was the most satisfactory fat for frying potato chips.

Porter, Michaelis, and Shay (*11*) concluded after frying doughs in animal fat, vegetable oil, and three hydrogenated vegetable fats, that the breaking down of fat involving the formation of acid was a direct result of a reaction with water at elevated temperatures. Up to approximately 0.75 percent acid the rate was considerably greater. Doughs fried in a hydrogenated vegetable fat between the temperatures of 176.5° to 199° C. had an objectionable flavor when about 2 percent of acid (as oleic) was present.

Coe (*3*) through extensive investigations has shown that oil-bearing foods subject to rancidity may be protected from deleterious wave lengths of light by enclosing them in green containers or wrappers of a proper shade. He has given a comprehensive review of the literature in relation to the rancidity problem.

MATERIALS AND METHODS

The following fats were used for the cooking tests:

Three kettle-rendered lards produced by the Bureau of Animal Industry at the National Agricultural Research Center of the United

States Department of Agriculture, Beltsville, Md., from animals fed on rations consisting largely of (1) peanuts, (2) corn, and (3) brewer's rice. These lards were prepared according to the tentative method adopted by the 1932 Conference on Cooperative Meat Investigations. They are referred to in this paper as "peanut", "corn", and "rice" lards.

The peanut, corn, and brewer's rice rations were used to give characteristically soft or oily, medium hard to hard, and very hard lards, respectively. Six representative commercial fats were used, namely, a standard prime steam lard (an unrefined product of the wholesale trade), a hydrogenated lard, a hydrogenated cottonseed oil, a highly refined corn oil, a highly refined cottonseed oil, and a highly refined peanut oil. The commercial fat samples were obtained directly from the manufacturers at about the time the lards were rendered at Beltsville.

All the fats were stored in 5-pound covered tin containers and kept at a temperature of from 4.4° to 7.5° C. throughout the experiment.

The potatoes were of the Green Mountain variety and were harvested from the Bureau of Plant Industry plots at Presque Isle, Maine, by October 1. They were held in storage at a temperature of 16° C. during the period of the experiment, since Sweetman (*14*) and Wright and his associates (*18*) had reported that potatoes stored at a lower temperature develop too much sugar to make a desirable potato chip.

The potatoes were washed and allowed to dry overnight. The blemishes were removed from a weighed quantity, then the unpared potatoes were sliced 1.5 mm in thickness by means of a rotary hand slicer. Four kilograms of potato slices were needed for the whole series of fryings made in 1 day. Approximately 500 g were sliced at one time, washed for 2 minutes, then dried thoroughly between towels and placed in tightly covered jars. When the entire 4 kg had been so treated, the slices were all mixed and returned to the jars.

For frying, 2 kg of each fat was heated to 185° C. in a 5-quart iron kettle 7.75 inches in diameter. The temperature was held stationary for one-half minute and then a wire basket containing 100 g of potato slices was lowered into the fat. The slices were stirred with a wire fork during the 2-minute frying period. The frying operation was continued in each fat until the 400-g sample of potato slices was fried. After the four fryings, each fat was strained through cheesecloth and placed in a covered pail in the refrigerator. All the fats were used for frying during 1 day, and the fryings were continued until 10 fryings had been made in each fat. As the quantity of each fat diminished a corresponding decrease was made in the quantity of potato slices used, so that the weight ratio of fat to slices was kept at 5 to 1. The potato chips were drained on absorbent paper, weighed, and stored in tightly covered jars. At the end of the tenth frying the experiment was repeated and fresh fat was used. The two sets of fryings are referred to as series 1 and series 2.

A record was kept of the temperature readings for each fat at the end of each half minute during the frying of the chips.

To make the moisture and fat determinations, samples of chips from each fat were ground in a mortar immediately after frying, and dried to constant weight at 100° C. The loss was calculated as mois-

ture. The dried material was extracted for 18 hours with anhydrous ether in a Soxhlet extraction apparatus and the fat content calculated. These determinations were repeated on the chips from the first and the tenth fryings.

The day after frying, the potato chips were judged by a committee of three persons representing the Bureaus of Home Economics and Plant Industry. The record card (fig. 1) included color, luster, aroma, crispness, oiliness, and flavor. Each judge also gave a general opinion in terms of very poor, poor, fair, good, and very good for all the chips included in the experiments.

Chemical and physical determinations, including those for peroxide value, iodine number, and free fatty acid (as oleic) were made on the

Record card for deep-fat frying

No. of Frying_____ Sample No._____ Product, Potato Chips

	Factor	3	2	1	Remarks
Color	Desirability	Desirable	Neutral	Undesirable	
Luster	Intensity	Bright	Moderately bright	Dull	
Aroma	Intensity	Slightly pronounced	Moderately pronounced	Very pronounced	
	Desirability	Desirable	Neutral	Undesirable	
Crispness	Intensity	Crisp	Moderately crisp	Slightly crisp	
Oiliness	Intensity	No oiliness	Moderately oily	Oily	
Flavor	Intensity	Slightly pronounced	Moderately pronounced	Pronounced	
	Desirability	Pleasant	Neutral	Unpleasant	

General Desirability: Very poor, poor, fair, good, very good.

Signature of judge

FIGURE 1.—Reproduction of record card used for recording the general quality of potato chips.

fats, both before and during the frying operations, to determine the extent of deterioration. In addition, free fatty acid was determined in the fats extracted from chips in the first and tenth fryings with each of the fats. The extraction was carried out under essentially identical conditions of time and temperature, ether being used as the solvent.

Thirty-gram samples of chips from the first frying in each fat were stored in white glassine bags at room temperature (22.6° C.) and in the refrigerator (3.2 C.), in each case in the absence of light. Organoleptic tests for rancidity were made by the judges at weekly intervals until rancidity developed in all chips stored at room temperature.

EXPERIMENTAL DATA

FRYING TEMPERATURE READINGS

Preliminary frying tests with the potatoes that were slightly high in sugar showed that the most desirable chips were produced by frying for 2 minutes at an initial temperature of 185° C. The average

temperature readings for the two series taken at half-minute intervals during the 2-minute frying period of the chips were: 0.5 minute, 161.1°; 1 minute, 152.2°; 1.5 minutes, 149.3°; 2 minutes, 148.5°.

MOISTURE AND FAT DETERMINATIONS

Analysis of the raw potatoes showed 22.8 percent dry matter and 0.038 percent fat. Analysis of the potato chips after the first and tenth fryings indicated an average range of 1.4 to 1.9 percent moisture for the chips fried in all the fats. The fat absorption by the chips was about the same for all kinds of fat, the average for the first frying of series 1 and 2 being 42.07 percent and for the tenth frying of the two series 43.6 percent. The maximum and the minimum fat absorption in the first frying of series 1 and 2 was 42.93 percent and 40.26 percent, respectively. The maximum and the minimum fat absorption in the tenth frying of series 1 and 2 was 45.05 percent and 42.81 percent, respectively.

PROGRESSIVE DETERIORATION OF FATS

CHEMICAL CHANGES

Determinations made on the fats (table 1) showed them to be reasonably fresh when first received. The lards, exclusive of the hydrogenated, showed higher values for free fatty acids than did the hydrogenated and refined oils. Analyses of the fats of both series after use showed increases in free fatty acid and in peroxide values, and decreases in iodine number. Generally speaking, these values indicated some deterioration through continued use of the fat.

TABLE 1.—*Free fatty acid, iodine number, and peroxide value of fats when unused and after use in 5 and 10 fryings of potato chips*

Kind of fat	Free fatty acid (as oleic acid)					Iodine number			Peroxide value		
	Un-used fat	Series 1		Series 2		Un-used fat	Series 1	Series 2	Un-used fat	Series 1	Series 2
		Fifth frying	Tenth frying	Fifth frying	Tenth frying		Tenth frying	Tenth frying		Tenth frying	Tenth frying
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*				*Milli-mols O₂ per kg*	*Milli-mols O₂ per kg*	*Milli-mols O₂ per kg*
"Rice" lard	0.43	0.70	0.88	0.54	0.68	54.1	50.4	51.6	1.5	7.3	12.8
"Corn" lard	.38	.51	.56	.54	.60	59.4	54.1	54.9	1.3	14.7	21.1
"Peanut" lard	.20	.29	.38	.28	.41	91.5	86.8	86.6	2.0	19.0	24.9
Prime steam lard	.40	.50	.58	.45	.54	65.2	59.6	60.4	.3	11.6	16.0
Hydrogenated lard	.03	.13	.24	.10	.19	62.0	56.5	57.8	1.0	14.3	12.3
Hydrogenated cottonseed oil	.04	.11	.18	.08	.17	69.6	64.2	64.9	.4	12.3	15.6
Cottonseed oil	.03	.08	.15	.06	.10		109.0	107.1	3.9	11.6	11.9
Corn oil	.05	.06	.14	.07	.14	128.2	125.1	121.8	4.6	10.1	11.3
Peanut oil	.13	.16	.26	.16	.23		91.4	92.9	2.0	15.6	25.4

The free fatty acid values of the unused fats varied with the source of the fats and the method used in their manufacture. After use in 10 fryings of potato chips the increase in free fatty acid was less in the oils and in the hydrogenated cottonseed oil than in the lards. Decrease in iodine number amounted to as much as 5.6 units (prime steam) among the lards in series 1 and to 6.4 units for the corn oil in

series 2. The increases in peroxide values showed no outstanding tendency for any fat or group of fats.

While the determinations made at the end of the fifth frying (table 1) showed increases in fatty acid over values found in the unused fats, there was no indication that they could be considered as midpoints for the determinations made after the tenth fryings.

COLOR OF THE FATS

A comparison of the color of the different fats before the first and after the tenth frying showed greatest discoloration in the lards, with the exception of the hydrogenated lard. The least deterioration as indicated by color was in the oils. The change was slight and uniform for all the oils.
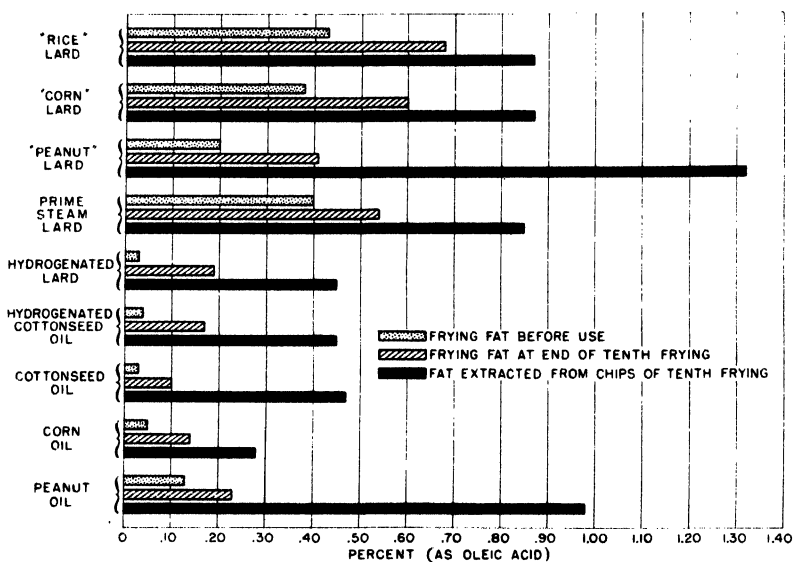
FIGURE 2.—Changes in free fatty acid content of nine fats used as the cooking medium for potato chips.

THE FAT IN THE CHIPS

As indicated by figure 2, the free fatty acid content of the fat extracted from the chips was higher in every instance than the fat in which the chips were fried.

PALATABILITY OF THE CHIPS

The palatability values given in table 2 for each series are means derived from 30 independent judgments, that is, 3 judgments on the ships from each of 10 fryings.

In order to evaluate the judgments the ranking given to the chips fried in the different fats was averaged for each quality factor (table 2). By using Fisher's method (4) of analysis of variance to determine the significance of difference between the means it was found that differences were significant in the intensity of luster, desirability of flavor, and the general desirability arrived at, in the judging of the chips fried in the different fats. Snedecor's method (12) was followed for the other items on the grading chart.

TABLE 2.—*Average of the mean grades of the palatability factors used in judging potato chips fried in 9 fats during 10 fryings*

Palatability factor on which chips were judged	"Rice" lard	"Corn" lard	"Pea-nut" lard	Prime steam lard	Hydro-gen-ated lard	Hydro-gen-ated cotton-seed oil	Cotton-seed oil	Corn oil	Peanut oil
Color, desirability:									
Series 1	1.9	2.0	2 3	2 5	2.3	2.3	2.5	2.3	2.6
Series 2	2 2	2.4	2.7	2.6	2.3	2 5	2 7	2 7	2.9
Average	2 08	2 21	2.51	2.58	2.33	2.41	2.58	2.50	2.73
Luster, intensity:									
Series 1	1.2	1.2	2.3	1.5	1.4	1.6	2 4	2.2	2 4
Series 2	1.1	1.2	2.2	1.7	1.1	1.3	2.2	2 2	2.2
Average	1.13	1.19	2.21	1.56	1.23	1.46	2.33	2.21	2.29
Aroma, intensity:									
Series 1	1.9	1.9	1.6	2.0	2.3	2.3	2 3	2 2	2.4
Series 2	1 8	1.7	1.9	1.6	2.0	2.3	2.3	2.1	2.3
Average	1.86	1.84	1.75	1.78	2.14	2.29	2.29	2 17	2.36
Aroma, desirability:									
Series 1	1.6	1 6	1.3	1.8	2.0	2.2	2 2	2 1	2.3
Series 2	1.6	1.5	1.6	1.4	1.7	2.0	2.1	2 0	2.2
Average	1.53	1.55	1.46	1.60	1.83	2.13	2.15	2.10	2.25
Crispness, intensity:									
Series 1	3.0	2 9	3.0	2.9	3 0	3.0	3.0	2.9	2.9
Series 2	3.0	3 0	3.0	2 9	3.0	3 0	3.6	3.0	3.0
Average	2 96	2.93	2.98	2.93	2 98	2 96	2 98	2.93	2 96
Oiliness, intensity:									
Series 1	3.0	2.9	2 4	2.8	3 0	3 0	2 6	2.5	2.4
Series 2	2 9	3.0	2.3	2 7	3 0	2 9	2 3	2 4	2.3
Average	2.95	2.91	2.36	2 76	2.98	2.95	2.44	2.43	2 36
Flavor, intensity:									
Series 1	1.6	1.6	1.9	1.6	1.9	2.3	2 2	2 2	2.2
Series 2	1.7	1.6	2.3	1.2	1.9	2.1	2 2	2 3	2.4
Average	1.61	1.56	2.08	1.41	1.88	2.20	2.23	2.23	2.30
Flavor, desirability:									
Series 1	1.4	1.4	1.7	1 5	1.7	2.3	2.1	2 3	2.4
Series 2	1.4	1.5	1 9	1.1	1.5	2.0	2.1	2 3	2.5
Average	1.43	1.50	1.78	1.31	1.61	2.11	2.11	2.30	2.46
General desirability:									
Series 1	2.3	2.2	2.7	2.4	2.8	3.5	3.7	3 4	3.8
Series 2	2.2	2.4	3.0	1.9	2.6	3.1	3.4	3.5	3.9
Average	2 25	2.28	2 85	2.13	2.68	3.35	3.55	3.45	3.88

Tests of significance of the differences between the means were made for series 1 and 2 separately and combined. The results of the two series were remarkably corroborative. Where any difference occurred in the relative positions of two fats in the two series, it was found that on the whole the difference in means between them was insignificant or unimportant. The factors graded by the judges were considered in the order given on the grading chart.

Peanut oil, cottonseed oil, prime steam lard, peanut lard, and corn oil as a group were found to impart the most desirable color to the potato chips. Hydrogenated cottonseed oil and hydrogenated lard formed an intermediate group; and corn and rice lard a lower group which gave the least desirable color in the chips.

In the average of the two series of tests, cottonseed oil imparted the brightest luster to the chips, but the differences among cottonseed, peanut, and corn oil, and peanut lard were unimportant. These four formed one group; prime steam lard and hydrogenated cottonseed oil, an intermediate group; and the other lards the lowest group, which gave less luster to the chips.

In determining intensity of aroma the chips with the most pronounced aroma were considered of lowest grade. In both series of tests, peanut oil appeared to give the least pronounced aroma to the chips, but the differences between its effect and the effect of the other oils were not significant. Hydrogenated lard stood midway, more pronounced than the oils but less pronounced than the other lards. The differences among the group consisting of rice, corn, prime steam, and peanut lard, which gave the most pronounced aroma to the chips, were insignificant.

Peanut oil in both series seemed to impart to the chips the most desirable aroma, yet statistically it shows no significant difference from the other oils. The four oils formed one group, giving the most desirable aroma to the chip, while hydrogenated lard was intermediate, being less desirable than the oils but more desirable than the other lards. Prime steam, rice, corn, and peanut lard formed a homogeneous group neutral to undesirable in effect on aroma.

A close relationship existed between intensity and desirability of aroma. The aroma considered most desirable was the least pronounced. A close positive association also existed between the desirability of flavor and the desirability of aroma imparted to the chips by the fats. The only exception was peanut lard, which imparted a pronounced and undesirable aroma, but the flavor given bordered on the neutral and was not so undesirable as that of the other lards.

There was no significant effect in the crispness of the chips according to kind of fat used for frying. An examination of the averages showed that all the potato chips had practically the same crispness.

There was little difference between the chips as to oiliness. Hydrogenated lard, hydrogenated cottonseed oil, rice lard, and corn lard as a group produced the least oily chips. Chips fried in prime steam lard were significantly more oily than those listed above, but less oily than chips fried in cottonseed, corn, and peanut oil, and peanut lard. There were no significant differences among the last four.

In regard to intensity of flavor, the chips fell into three groups. The oils imparted a less pronounced flavor than the other two groups but were not significantly different from each other. Peanut lard and hydrogenated lard gave a more pronounced flavor than the oils but less pronounced than the other lards; and rice, corn, and prime-steam lard, gave the most pronounced flavor but were not significantly different from each other.

In both series of tests peanut oil was judged to impart the most desirable flavor to the chips. Corn oil came second to peanut oil in both series, and the two cottonseed oils third and fourth. Peanut lard and hydrogenated lard gave a less desirable flavor to the chips than the oils but a more desirable flavor than corn, rice, or prime-steam lard. The last three gave the least desirable flavor but were not significantly different from each other in their effect.

The judges placed the chips fried in the different fats, in respect to general desirability, in five grades: very poor, poor, fair, good, and very good.

Desirability of flavor was evidently the most important item in determining the general grade. For the two series combined, the fats occupied the same relative positions as to the general desirability of the chips fried in them that they did for desirability of flavor of the chips with one exception, namely, in general desirability cottonseed oil came after peanut oil. Its average for effect on flavor was identical with that of hydrogenated cottonseed oil and below corn oil, although the difference was not significant.

The chips cooked in oils were preferred on the whole to those cooked in the lards. Of the lards the peanut and the hydrogenated gave better general results than the corn, rice, or prime steam. The
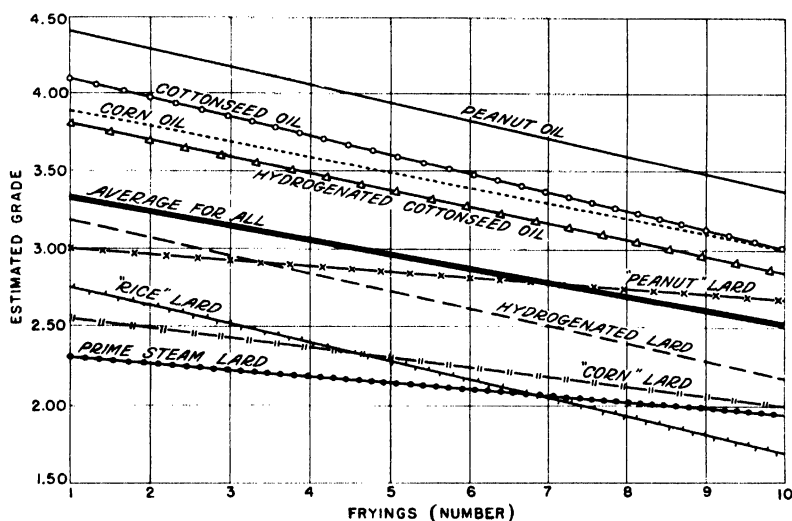
FIGURE 3.—Regression lines showing the estimated loss of grade for general desirability of chips fried in each of the nine fats during the 10 fryings of the two series combined.

differences among the last three were unimportant. An unweighted average for all eight factors, excluding general desirability, placed the fats in the same relative positions with the exception of prime steam lard, which ranked above corn and rice lard. This was evidently due to the influence of the score for color, as prime steam lard as well as cottonseed oil was second highest. As stated before, however, tests of significance indicated that the slight differences between the means of the prime steam, corn, and rice lards were probably due to chance and that the judges were unable to discern any consistent difference among them. It may be considered that the judgments for general desirability gave an accurate estimate of general effect of the fats on the chips.

CHANGES IN FATS DURING USE AS EVIDENCED IN THE CHIPS

There was some deterioration in the general quality of the chips as the number of fryings increased (fig. 3). Computations made after the chips had been judged for general desirability, showed that the

corn-lard, prime-steam-lard, and peanut-lard chips fell 0.04 to 0.06 of a grade per frying, and that all of the chips fried in the other fats fell 0.10 to 0.12 of a grade. The number of times the fats were used up to 10 did not affect the relative desirability of the chips materially. Chips fried in rice lard, which were in general superior to those fried in corn and prime-steam lard at the beginning of the tests, fell more rapidly in quality than the chips fried in the other lards, so that after the fifth frying they were inferior to those fried in corn lard, and after the seventh frying they were inferior to those fried in prime-steam lard. Chips fried in hydrogenated lard, which at the beginning were judged to be superior to those fried in peanut lard, were inferior to them after the third frying . Chips fried in cottonseed oil at the beginning were slightly superior to those fried in corn oil, but at the end of the tenth frying no difference could be discerned. Chips fried in peanut oil, which were held to be superior at the beginning of the tests, had about the same relative quality at the end of the tenth frying.
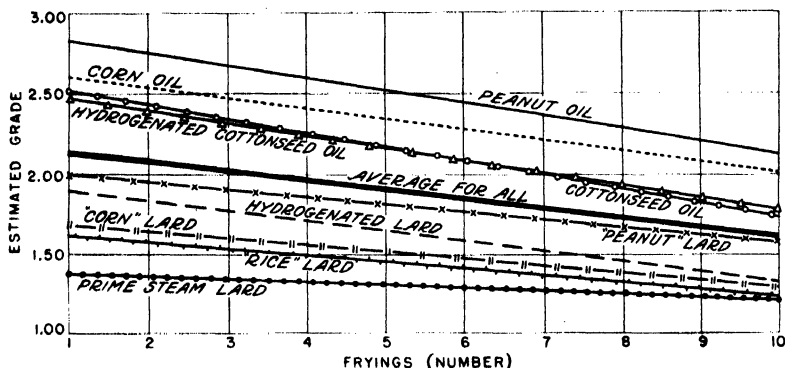
FIGURE 4.—Regression lines showing the estimated loss of grade for desirability of flavor of chips fried in each of the nine fats during the 10 fryings of the two series combined.

There was also a decrease in the desirability of flavor of the chips as the number of fryings increased (fig. 4). This trend was as follows: Cottonseed oil, peanut oil, and hydrogenated cottonseed oil fell 0.08 to 0.09 per frying; corn oil, hydrogenated lard, 0.06 to 0.07; peanut lard, rice lard, and corn lard, 0.04 to 0.05; prime-steam lard, 0.02. The flavor of the chips fried in the oils deteriorated somewhat more rapidly than those fried in the lards. However, at the end of the 10 fryings the chips fried in oil were still superior, although there was a slight tendency for the grades for flavor of the chips to converge as the fats were used repeatedly.

EFFECT OF STORAGE ON THE POTATO CHIPS

Organoleptic tests of chips fried in each of the fats and stored without light in white glassine bags at average room temperature and in a refrigerator indicated that low temperatures in most cases retarded the development of organoleptic rancidity (table 3). The storage temperature did not affect the crispness of the chip during the 60-day storage period.

TABLE 3.—*Effect of storage temperature on development of organoleptic rancidity and free fatty acids in potato chips fried in nine fats*

Fat used to fry potato chips	Period required for development of rancid flavor in chips when stored at—		Free fatty acid (as oleic acid)	
	Room temperature (22.6° C.)	Refrigerator temperature (3.2° C.)	Room temperature (22.6° C.)	Refrigerator temperature (3.2° C.)
	Days	*Days*	*Percent*	*Percent*
"Rice" lard	32	39	0.80	0.70
"Corn" lard	32	32	.49	.54
"Peanut" lard	25	39	.39	.34
Prime steam lard	32	39	.60	.52
Hydrogenated lard	32	39	.20	.18
Hydrogenated cottonseed oil	32	39	.19	.18
Cottonseed oil	32	46	.19	.15
Corn oil	46	46	.18	.15
Peanut oil	60	60	.24	.24

DISCUSSION OF RESULTS

In the present investigation low fat absorption was regarded as one of the criteria of a satisfactory fat for frying potato chips. Although the quantity of fat absorbed by the experimental chips was 6.9 percent higher than that reported by Woodruff and Blunt (*17*) and 7.3 percent higher than that reported by Atwater and Bryant (*1*) for commercial chips, the results agree with those of Woodruff and Blunt in that they were about the same for each of the different fats. Such findings are significant because the fats included those of both animal and vegetable origin, as well as those manufactured by different methods. The high fat absorption of the chips in this study may have been due to the lower frying temperature necessitated by the high sugar content of the potatoes.

From the standpoint of economy, a long frying life in a fat is desirable. The theory has been advanced (*9, 17*) that it is largely the long heating of fats which brings about their deterioration. The experimental fats were all heated to a temperature of 185° C. during the 10 fryings which totaled 8.5 hours, but the actual frying time was only 80 minutes. While none of the fats after 10 fryings showed a high free fatty acid content, the peroxide values were high enough, especially in the case of the lards, to make their fitness for use questionable. The large increase in free fatty acids in the fats extracted from chips of the tenth frying likewise brings into question the suitability of these fats and to some extent accounts for the poorer quality of the chips. There was no significant difference in the rate of deterioration of the fats from the first to the tenth frying, as shown by the judgments on the chips from each successive frying.

Frying fats are known to show an increase in acidity during use (*2, 5, 10, 11, 17*). The maximum amount (0.88 percent) found in this experimental work was well below the 2.0 percent at which objectionable flavor was reported by Porter, Michaelis, and Shay (*11*) when frying doughnuts.

A comparison of the chemical tests made upon the fats with judgments concerning the desirability of flavor of the chips showed that little if any relationship existed. While the chemical tests were consistent in showing the deterioration of a single fat they were of

little value in showing the differences between the kinds of fats. The different methods of manufacture may have had some bearing upon these findings (*17*).

King, Roschen, and Irwin (*8*) in their peroxide determinations, used a value of 20 millimols per kilogram as the point at which incipient organoleptic rancidity occurred in lards subjected to their accelerated stability test. They further noted that the rancid point was higher in the case of corn and cottonseed oils and of cottonseed-oil shortenings than in lards. These differences in the relation of rancid taste or organoleptic rancidity to peroxide content are probably due to variations in the relative speeds of the two reactions concerned in the development of rancidity (1) peroxide development and (2) formation of the compounds contributing to rancid flavor, discussed by Kilgore (*7*). It is probable that this variation in the relative speed of the two reactions is affected not only by the kind of fat but by the method of handling, and that it would be very much influenced by variations in the cooking process. Because of this it is impossible to state definitely the peroxide value at which any fat becomes rancid. It is likewise not possible to use the peroxide value as a basis for ranking the various fats in the present study as to their degree of rancidity. The general increase in peroxides showed that all the fats were in the process of developing rancidity. The differences between the two groups based on origin (animal or plant) appeared no greater than the differences within groups.

SUMMARY

Nine fats, including three kettle-rendered lards from animals fed on rations consisting largely of (1) peanut, (2) corn, and (3) brewer's rice, a standard prime steam lard, a hydrogenated lard, a hydrogenated cottonseed oil, and three highly refined oils from corn, cottonseed, and peanuts, respectively, were studied for use in the frying of potato chips. Frying technique was modified to permit of operations under controlled conditions. The results showed that fat absorption in the potato chips was about the same for all the fats used.

The chemical tests showed deterioration in all the fats. After 10 fryings, none of the fats had a high free fatty acid content, but the peroxide values were high enough, especially in the case of the lards, to make their fitness for use questionable. The percentage of free fatty acids in the fat extracted from the chips was greater than in the frying fat. No relationship was found between the chemical changes and the flavor of the chips.

The averaged values of the palatability tests made upon the potato chips showed that from the standpoint of general desirability, the oils were preferable for frying to the lards. Desirability of flavor was the most important item in forming the general opinion, and in the tests made peanut oil was judged to impart the most desirable flavor to the chips. In general desirability, cottonseed oil ranked next to peanut oil. Of the lards, peanut and hydrogenated were the best for frying chips. Further analysis indicated that the slight differences between the desirability of prime steam, corn, and rice, lards for frying were not significant.

Potato chips fried in the oils and in most cases those stored in the refrigerator kept fresh for the longest period.

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FASCIATION OF SWEET PEAS CAUSED BY PHYTOMONAS FASCIANS N. SP. [1]

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INTRODUCTION

The cause of fasciation in winter-flowering sweet peas (*Lathyrus odoratus* L.) has been studied because the continued loss from this disease in Ohio greenhouses has created a request for control measures. Fasciation has been reported by Brown (*1*)[2] as occurring near Washington, D. C., and by Muncie and Patel (*2*) in Iowa, who attribute its cause, respectively, to a weak or highly specialized strain of *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. and to environmental or nutritional factors. This lack of agreement and the possibility that more than one agent or set of conditions might induce the disease suggested the advisability of investigating the cause in Ohio before seeking control measures.

SYMPTOMS

Many short, fleshy, thick, and aborted stems with misshapen leaves develop at or below the soil line on affected plants (fig. 1, *A*). These fasciated shoots originate either at the first or cotyledonary node, or at the second node of the stem (fig. 1, *B* and *C*). The mass of fasciated growth on old plants resembles a witches' broom and may reach a diameter of 3 inches, but usually does not extend over 1 or 2 inches above the ground; a normal green color develops in the portion exposed to the light. The main stem of an affected plant apparently grows normally, except that it is dwarfed and its productivity from the standpoint of marketable blossoms is reduced. Diseased plants seem to live about as long as normal individuals.

Certain symptoms, which are not usually found on naturally infected plants, may develop after artificial inoculation; for instance, if the young epicotyl is inoculated just as it emerges from the seed coat or if the organism is present in large numbers in the soil, a gall-like growth is produced sometimes and no main stem develops (fig. 1, *E*). When inoculations are made in the leaf axils of young plants some proliferation is evident after a month. The growth usually has the appearance of a multiple bud and occasionally one or more abnormal shoots will develop (fig. 1, *D*).

The writer has been unable to produce similar symptoms by inoculating sweet pea plants with the crown gall organism. Typical crown galls developed in the leaf axils (fig. 1, *F*) and on the lower part of the stem (fig. 1, *G*) after inoculations were made with *Phytomonas*

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tumefaciens.   Muncie and Patel (*2*) have reported similar results.
Smith (*5*), however, has pointed out that fasciation can be produced
by inoculating *P. tumefaciens* into the leaf axils of a variety of plants.

ISOLATION AND INOCULATION STUDIES

Some preliminary experiments were made to find out whether or
not the disease is infectious as it occurs in Ohio.   Fifty seeds of the
Ball's Orange variety of sweet peas were treated in a 5-percent solu-
tion of formaldehyde for 5 minutes and rinsed in sterile water.
Twenty-five were planted in 3-inch pots of sterilized soil, five to a pot.
The other 25 were rubbed in the crushed fasciated tissue from the
base of a diseased plant and then planted in pots of sterile soil.
Twenty plants grew in the control lot, and 6 weeks after planting all
20 were healthy; whereas 8 of the 22 plants from the seed rubbed in
the crushed tissue showed fasciation.

Formaldehyde-treated seed was planted in soil taken from a green-
house bed where most of the sweet peas were fasciated.   Another
portion of the same soil was sterilized by autoclaving before the seed
was planted.   Thirteen plants, eight of which showed fasciation 6
weeks after planting, grew in the unsterilized soil; whereas 12 plants,
1 of which was fasciated, grew in the sterilized soil.

These two preliminary tests, supplemented by observations in
commercial greenhouses where sweet peas were grown, seemed to
indicate that the disease in some instances at least was infectious.
Since Brown (*1*) had reported that fasciation of sweet peas may
be caused by the crown gall bacterium, an effort was made to isolate
this organism from sweet peas showing the disease.   The dilution
plate method was used with potato-dextrose agar, nutrient agar, and
Patel's (*3*) medium.   The plates were incubated for 3 or 4 days,
and all colonies resembling *Phytomonas tumefaciens* were transferred
and used in inoculation tests.   Numerous attempts were made during
1933 and 1934 to isolate the crown gall organism from fasciated
sweet peas, and about 80 selected colonies were tested for patho-
genicity.   In all instances negative results were obtained.   Occa-
sionally, fasciated plants developed but they appeared just as often
among the control plants as among those which had been inoculated.

In these early experiments a particular lot of seed was used which
always produced some fasciated plants, even though the seed was
treated by shaking for 5 minutes in a 5-percent solution of formalde-
hyde.   The seed coat of most varieties of sweet peas is wrinkled
and more or less pitted.   Numerous small air bubbles arise from
these pits and cling to the seed when it is submerged in a water
solution of a disinfectant, indicating that the surface is not thoroughly
wetted.   In the later inoculation tests the seed was immersed in
alcohol for 1 minute and then in 1–1,000 $HgCl_2$ for 20 minutes.
After this treatment, the control plants were always free of fascia-
tion.   The disease is probably seed-borne to some extent.

Finally, in a set of potato-dextrose agar plates which had been
incubated for 1 week, a few orange-colored bacterial colonies about
1 mm in diameter were noticed.   Since these were distinctly differ-
ent from any colonies observed previously, they were transferred to
agar slants and to broth.   On July 31, 1934, this organism, along
with several others which had been saved, was used to inoculate

FIGURE 1.—*A*, Sweet pea plant 3 months old showing fasciation; *B*, fasciation originating at the cotyledonary node; *C*, fasciation originating at the second node; *D*, 1 month after inoculation at leaf axil; *E*, epicotyl develops into a gall-like structure and sometimes no main stem forms if the organism is very abundant in the soil; *F*, result of inoculation at leaf axil with *Phytomonas tumefaciens*; *G*, result of inoculation on lower part of stem with *P. tumefaciens*.

seed of the Ball's Orange variety. Inoculations were made by pouring 24-hour-old broth cultures of the organisms over the seeds when they were planted. On September 4 the experiment was discontinued, and three plants out of five in the pot where the seed was inoculated with the orange bacterium showed fasciation. No fasciated plants occurred in any of the other pots, and of a total of 10 control plants all were normal. Apparently the same organism that had been used to inoculate the seed was reisolated from the fasciated plants.

This same organism has been isolated by the poured-plate method from diseased peas found growing in six different Ohio greenhouses. The inoculation and reisolation tests with these six isolates are summarized in table 1. The best results in making isolations have been obtained by taking a large piece of tissue from the base of the fasciated growth, dipping it in alcohol, flaming it, and then crushing it in about 5 cc of sterile water. After 2 to 4 hours one drop of the liquid is transferred with a sterile pipette to a sterile dish, two drops are placed in a second dish, and three drops in a third. Another set of three plates is prepared in exactly the same way. The plates are poured with melted potato-dextrose agar and incubated at 25° C. After 7 days the orange-colored colonies, about 1 mm in diameter, are easily detected. Sometimes as many as 10 to 12 appear in a single plate, more often the number is from 1 to 5, and in some plates none develop.

TABLE 1.—*Results of inoculating sweet peas* [1] *with poured plate cultures of the fasciation-producing bacteria*

Date of inoculation	Source of culture	Inoculations [2]	Fasciated	Controls [3]
1934		*Number*	*Number*	*Number*
Aug. 25	Wooster, Ohio, culture A [4]	18	12	20
Sept. 18	Wooster, Ohio, culture A1	24	23	25
Dec. 10	Reisolation of culture A1	10	3	17
Oct. 29	Wooster, Ohio, culture B [5]	5	1	5
Do	Wooster, Ohio, culture B1	5	4	5
Dec. 10	Reisolation of culture B1	11	4	17
Oct. 29	Barberton, Ohio, culture C	4	3	5
Do	Barberton, Ohio, culture C1	2	1	5
Dec. 10	Reisolation of culture C1	11	5	17
Oct. 29	Wooster, Ohio, culture D [6]	5	1	5
Do	Wooster, Ohio, culture D1	6	3	5
Dec. 10	Reisolation of culture D1	10	4	17
Nov. 8	Medina, Ohio, culture E	5	4	5
Do	Medina, Ohio, culture E1	5	3	5
Do	Medina, Ohio, culture E2	5	2	5
Do	Medina, Ohio, culture E3	5	3	5
Dec. 10	Reisolation of culture E	10	7	17
Do	Reisolation of culture E1	9	4	17
Nov. 14	Ashland, Ohio, culture F	3	1	5
Do	Ashland, Ohio, culture F1	5	1	5
1935				
Apr. 8	Reisolation of culture F1	6	3	30

[1] Variety, Ball's Orange.
[2] Inoculations were made by pouring a broth culture 24 to 48 hours old over the seeds at the time they were planted. 5 seeds were planted in 3-inch pots of sterilized soil placed on a greenhouse bench. Each pot rested on a block of wood to prevent contamination from 1 pot to another by drainage water. Pots and soil were sterilized by autoclaving.
[3] None of the controls became fasciated.
[4] From horticultural greenhouse.
[5] From botanical greenhouse.
[6] From Dewitt greenhouse.

Single-cell isolates [3] were made from all six of the original cultures. The methods employed in isolating and cultivating the single bacterial cells were essentially those described by Wright, Hendrickson, and Riker (7). All 15 of the single-cell isolates were tested for pathogenicity, and the results are summarized in table 2.

The organism was obtained from many of the plants inoculated with reisolations from diseased plants which had been inoculated with original cultures. These reisolates appeared to be identical with the original isolates when grown in pure culture. This fact, together with the results of inoculations given in tables 1 and 2, is thought to be abundant proof that the organism is pathogenic and capable of causing fasciation in sweet peas. It is believed to be an undescribed species.

TABLE 2.—*Results of inoculating sweet peas with single-cell isolates of the fasciation-producing bacteria*

Date of inocu-lation	Source of culture [1]	Inocula-tions [2]	Fasci-ated	Con-trols [3]
1935		*Number*	*Number*	*Number*
	Culture A A, single-cell isolate from culture A1	9	6	30
	Culture A A1, single-cell isolate from culture A1	8	6	30
	Culture B B, single-cell isolate from culture B1	8	6	30
	Culture C C, single-cell isolate from culture C1	5	2	30
	Culture C C1, single-cell isolate from culture C1	11	8	30
	Culture C C2, single-cell isolate from culture C1	7	2	30
	Culture C C3, single-cell isolate from culture C1	6	5	30
Apr. 8	Culture C C4, single-cell isolate from culture C1	6	3	30
	Culture D D, single-cell isolate from culture D1	5	1	30
	Culture D D1, single-cell isolate from culture D1	4	0	30
	Culture E E, single-cell isolate from culture E	7	3	30
	Culture F F, single-cell isolate from culture F1	21	7	30
	Culture F F1, single-cell isolate from culture F1	7	1	30
	Culture F F2, single-cell isolate from culture F1	11	5	30
	Culture F F3, single-cell isolate from culture F1	9	5	30
May 8	Culture D D1, single-cell siolate from culture D1	10	7	30
	Reisolation of culture A A, inoculation of May 8 [4]	7	2	18
July 3	Reisolation of culture A A1, inoculation of May 8 [4]	6	5	18
	Reisolation of culture B B, inoculation of May 8 [4]	11	10	18
Oct. 2	Reisolation of culture B B, inoculation of July 3	40	23	45
	Reisolation of culture C C, inoculation of May 8	9	1	18
	Reisolation of culture C C1, inoculation of May 8	8	2	18
	Reisolation of culture C C3, inoculation of may 8	7	4	18
July 3	Reisolation of culture C C4, inoculation of May 8	6	5	18
	Reisolation of culture D D1, inoculation of May 8	8	6	18
	Reisolation of culture F F, inoculation of Apr. 8	10	9	18
	Reisolation of culture F F1, inoculation of May 8	11	10	18

[1] See table 1.
[2] The inoculations were made from broth cultures 48 hours old by using sterile pipettes drawn to a sharp point. The seedlings (4 or 5 days old) were pricked at the cotyledonary nodes with the pipettes while drops of the inoculum flooded the area. Controls were treated in the same way except that sterile water replaced the inoculum.
[3] None of the controls became fasciated.
[4] The May 8 inoculations are not given, since they were a duplication of those of Apr. 8 and the results were similar.

HOST RANGE

No systematic effort has been made to determine the host range of the organism, but the results obtained by inoculating a few different plants indicate that susceptibility is not confined to a single genus. Twenty-five garden pea seeds (variety Premium Gem) were inoculated at the time of planting in a flat of sterilized soil. Nineteen plants grew, and 1 month later 17 showed symptoms of fasciation (fig. 2, *A*). The organism was recovered from these plants and used

to inoculate sweet peas which later became diseased. The writer has never observed fasciation on garden peas growing out of doors, but Brown (*1*) reported that a few plants of this kind affected with the disease had been sent to Washington from Maryland and Virginia.

Twenty-five seeds each of 10 commercial varieties[4] of winter-flowering sweet peas were sterilized and inoculated at the time of planting in a flat of sterilized soil. Five weeks later the plants were removed and examined for fasciation. All 10 varieties were found

FIGURE 2.—*A*, Garden pea (variety Premium Gem) 1 month after inoculation; *B*, fasciation at the base of petunia plant developing after inoculation; *C*, chrysanthemum naturally infected.

susceptible to the disease to about the same extent. An average of 64 percent of the plants was fasciated.

A mass of fasciated shoots developed at the base of petunia (fig. 2, *B*) tobacco (variety Quesnel), and *Gypsophila paniculata* plants after inoculation by the needle-prick method. The organism was isolated from a fasciated growth at the base of a geranium from Canton, Ohio, and from a chrysanthemum received from Dr. R. P. White of New Jersey. Geranium plants were later inoculated with the organism and developed symptoms of the disease. Many plants of several varieties of chrysanthemums in one Ohio greenhouse were fasciated

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[4] Ball's Orange, Ball's Rose, Harmony, Ball's Blue, New Majestic Rose, New Hope, Jeanne Mamitsch, Snow Storm, Mrs. Herbert Hoover, and White Harmony.

at the base when they were removed from the beds (fig. 2, *C*). The organism was isolated from some of these plants, and in this particular greenhouse fasciation has been especially common on winter and spring crops of sweet peas following chrysanthemums.

In a single experiment potato, tomato, aster, verbena, calendula, gaillardia, snapdragon, canterbury-bell, zinnia, marigold, and bean plants were inoculated at the base of the stem but negative results were obtained.

THE CAUSAL ORGANISM[5]

Unless otherwise indicated the methods given by the Society of American Bacteriologists (*6*) have been followed in making the determinations described below. Each of the tests was run in duplicate or triplicate. In most cases the tests were made three different times, usually with all 15 of the single-cell isolates and some of the original cultures.

CULTURAL CHARACTERISTICS

Three of the six original isolates were of the rough colony type, whereas the other three were smooth. Six of the fifteen single-cell isolates were of the rough type and have remained so in culture. Both rough and smooth types were obtained from the same culture in one instance when single cells were isolated. Rough colony types have been isolated from plants inoculated with a single-cell isolate which has consistently been the smooth type in culture.

The cultural characteristics of the organism grown on and in several culture media incubated at a temperature near 25° C. were as follows: On potato-dextrose agar plates minute, light cream-colored colonies were visible on the surface after 72 hours. After 7 days the colonies were mostly punctiform, but some were circular with the largest not over 3 or 4 mm in diameter. Submerged colonies were barely visible. The color after 1 week was cadmium yellow to deep chrome or orange buff (*4*). Rough colonies were near cadmium yellow, whereas the smooth type was more nearly deep chrome or orange buff.

On nutrient agar [6] plates the growth was much slower than on potato-dextrose agar, and the color was cream.

After 3 days on potato-dextrose agar slants the growth was moderate, filiform, raised, dull to glistening, and rugose in the case of rough colonies, glistening and smooth with smooth colonies, opaque, cadmium yellow to deep chrome in color, and butyrous in consistency. No odor was detected and the medium was not changed in appearance. After a week the characteristics were the same except that abundant growth was present.

On nutrient agar slants after 1 week the growth was moderate, filiform, flat, dull to glistening, smooth, opaque, cream-colored, and butyrous. There was no visible change in the media and no odor.

Scarcely any clouding of nutrient broth [7] was visible after 24 hours, but after 48 hours broth was slightly clouded and a thin, fragile pellicle with a distinct rim had formed.

After 7 days in Fermi's solution the medium was slightly clouded and a sparse, fragile pellicle with a rim had formed on the surface.

Uschinsky's solution was slightly clouded after 7 days, a pellicle similar to that in Fermi's solution had formed, but the growth was a little more abundant than in the latter.

In Cohn's solution growth was not as abundant after 7 days as in either Fermi's or Uschinsky's medium. Scarcely any clouding was visible, but in most instances a very fragile pellicle had formed.

MORPHOLOGY

When grown on potato-dextrose agar the organism was a rod with pointed to rounded ends, usually growing singly but sometimes in pairs or even short chains of three or four cells. Occasionally a pair of cells, probably in the act of dividing, formed a U or V. Grown on this medium for 18 to 24 hours at 25° C. and stained with Gram's stain, the size was 1.5μ to 4.0μ long and 0.5μ to 0.9μ wide. The average measurement of 50 individuals was 2.7μ by 0.69μ. This average was obtained by measuring 10 individuals of each of 5 strains by the use of a filar ocular micrometer.

No evidence of motility has been observed on potato-dextrose agar or in peptone-salt-dextrose liquid medium. Many hanging drops have been prepared and studied in addition to those observed while making single-cell isolates. Flagella could not be demonstrated with Casares-Gil's flagella stain.

Cultures 24 and 48 hours and 5, 8, and 42 days old growing on potato-dextrose agar were examined for capsules by Anthony's method. The formation of capsules could not be demonstrated in any of the rough colony type of single-cell isolates. In the smooth type, however, what appeared to be capsules were found in the cultures which were 8 and 42 days old. Younger cultures of this type showed no evidence of capsules.

Attempts to demonstrate spores in potato-dextrose agar cultures which were 3, 10, and 12 weeks old gave only negative results. Heavy suspensions of the organism were prepared in sterile water and heated to 85° C. for 10 minutes in glass ampules. No growth developed when transfers were made to nutrient broth. In material from cultures which were 1, 2, 4, and 8 weeks old stained by Dorner's method, no spores were found. One, two, and sometimes three small round bodies, however, were observed in the bacterial cells.

PHYSIOLOGICAL CHARACTERS

LIQUEFACTION OF GELATIN.—No liquefaction occurred in gelatin stabs incubated at 20° C. for 7 weeks. In the same tests *Phytomonas phaseoli* (E. F. S.) Bergey et al. used for comparison produced strong liquefaction. A negative test for liquefaction of gelatin was also obtained by using Frazier's gelatin medium. *P. phaseoli* gave a positive test, whereas a negative test was obtained with *Phytomonas stewarti* (E. F. S.) Bergey et al. by this method.

LITMUS MILK.—After 48 hours in litmus milk slight bluing occurred at the top of the milk column. The blue color was distinct after 5 days and the litmus was slightly reduced in the bottom of 16-mm tubes containing about 8 cc of the medium. At the end of 1 month the milk was blue to within one-half inch of the bottom of the tubes where the litmus was reduced. No curd formed and the milk appeared unchanged except as noted. A pellicle consisting of

clumps of the organism, cadmium yellow in color, formed on the surface. The milk in the control tubes after 1 month was a uniform lavendar throughout.

REDUCTION OF NITRATES.—Nitrates were reduced to nitrites. Tests were made on synthetic nitrate agar and on peptone beef-extract nitrate agar by using sulphanilic acid and alpha-naphthylamine reagents. *Escherichia coli* (Escherich) Castellani and Chalmers and *Phytomonas phaseoli* were used for comparison. After 24 hours a faint but distinct test for nitrites was obtained in the synthetic medium. After 48 hours and after 1 week nitrites were found in the synthetic nitrate agar, but no evidence of nitrites appeared in the peptone beef-extract nitrate agar. In no case was the red color as intense in the tubes of synthetic medium inoculated with the organism in question as with *E. coli*. In every test with both media the control tubes, as well as those inoculated with *P. phaseoli*, showed no evidence of nitrites.

Ammonia was not found by the Hansen test in synthetic nitrate agar but ammonia was present in cultures on peptone beef-extract nitrate agar after they had reached an age of 2 and 7 days.

INDOLE PRODUCTION.—No indole was produced. Bacto-tryptone cultures 2, 4, 12, and 28 days old all gave negative tests by the Goré modification of the Ehrlich-Böhme method. A positive test for indole was obtained with *Escherichia coli* and a negative test with *Phytomonas phaseoli*.

FERMENTATION OF CARBOHYDRATES.—Three different tests were made with each carbon source. (1) Synthetic carbohydrate medium[8] with bromcresol purple indicator was prepared so that it contained 1 percent of the carbon source and 1.5 percent of agar. The carbon source was added before autoclaving. (2) The synthetic carbohydrate medium without agar was tubed in test tubes containing Durham tubes and sterilized. The carbon source, if soluble, was sterilized separately by passing through a Chamberland-Pasteur L3 filter under a suction of 5 to 10 inches of mercury. It was then transferred aseptically with pipettes to the tubes of medium in amount sufficient to bring the concentration to 1 percent. Media prepared in this way were incubated for 1 week before using, and if any tubes showed contamination they were discarded. (3) Synthetic carbohydrate liquid media were prepared in the manner just indicated, except that bromcresol purple indicator was added.

An increase in the hydrogen-ion concentration was detected in the case of the indicator media by the change in color of the indicator as compared to uninoculated control tubes. Any change in the hydrogen-ion concentration of the nonindicator media was determined by comparing the pH of inoculated with uninoculated tubes of the media. The pH measurements were made electrometrically with a potentiometer, quinhydrone electrode, and calomel half cell.

All 15 of the single-cell isolates, as well as some of the original isolates, were used with the less expensive carbon sources. With the more expensive sugars the tests were made with only five of the single-cell isolates.

No gas appeared in the Durham tubes in the liquid cultures with any of the carbon sources.

[8] Monobasic ammonium phosphate, 1.0 g; potassium chloride, 0.2 g; magnesium sulphate, 0.2 g; water, 1,000 cc; carbon source, 10.0 g.

The hydrogen-ion concentration was increased in media containing dextrose, sucrose, maltose, dextrin, glycerol, mannitol, arabinose, xylose, galactose, mannose, and levulose; whereas the reaction did not change when lactose, inulin, rhamnose, raffinose, and starch were used as sources of carbon. In no instance were the results different when the carbon source was autoclaved than when it was sterilized by filtration.

The hydrogen-ion concentration produced from different carbon sources by five of the single-cell isolates after an incubation of 1 week in synthetic carbohydrate medium is given in table 3.

TABLE 3.—*Hydrogen-ion concentration produced from various carbon sources by single-cell isolates of the fasciation-producing bacteria after an incubation of 7 days in synthetic carbohydrate media at room temperature*

Source of carbon	AA	CC4	DD	EE	FF	Control
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
Dextrose	6.8	6.7	6.65	6.85	6.65	7.0
Sucrose	6.5	6.25	6.15	6.3	6.2	6.95
Maltose	6.65	6.65	6.65	6.65	6.5	6.9
Dextrin	6.65	6.55	6.6	6.65	6.65	6.7
Glycerol	6.7	6.6	6.7	6.7	6.7	6.9
Mannitol	6.5	6.5	6.5	6.4	6.6	6.9
Arabinose	6.45	6.1	6.5	6.35	6.35	6.9
Xylose	6.7	6.7	6.8	6.75	6.85	7.0
Galactose	6.8	6.7	6.8	6.9	6.8	7.0
Mannose	5.6	5.4	4.5	5.6	4.5	6.9
Levulose	6.85	6.65	6.5	6.7	6.7	7.0
Lactose	6.75	6.85	6.8	6.85	6.8	6.85
Inulin	6.7	6.7	6.7	6.7	6.7	6.7
Rhamnose	6.95	6.95	7.0	7.0	7.0	7.0
Raffinose	6.85	6.85	6.85	6.85	6.85	6.85
Starch	7.0	7.0	7.0	7.0	7.0	7.0

DIASTATIC ACTION.— Starch was not reduced. On starch-agar plates streaked with the organism and incubated for 7 days at 25° C. no clearing was evident when the plates were flooded with iodine.

HYDROGEN SULPHIDE PRODUCTION.—Hydrogen sulphide was produced. All single-cell isolates were used to inoculate Difco lead acetate agar slants and lead acetate papers were suspended in the tubes. After 3 days very little change could be detected in the agar, but the test papers were blackened at the tip in all instances and as much as 1 inch back from the tip in some cases. The organism produced more H_2S than was formed by *Phytomonas phaseoli*, as was indicated by the extent of the blackening of the test papers.

TOLERATION OF SODIUM CHLORIDE.—The organism grew in nutrient broth containing sodium chloride up to 8 percent. Ten percent practically inhibited growth. The growth in broth containing 6, 7, and 8 percent of sodium chloride consisted almost entirely of a fragile pellicle on the surface and a rim on the walls of the tube.

OXYGEN RELATIONS.— The organism was aerobic. In shake cultures a heavy growth developed on the surface of potato-dextrose agar, but no colonies formed in the agar at depths greater than one-eighth of an inch of the surface. When long sterile cover slips were placed on agar plates streaked with the organism no growth occurred under the cover except at the edge. Melted agar suspensions of the organism were drawn into sterile glass tubes which were then sealed. Very weak growth, which was light in color, occurred at the ends of the agar column but none at all in the agar. When potato-dextrose agar

in bottles which could be sealed with airtight caps was inoculated, the growth was weak and light in color in contrast to the heavy growth of cadmium yellow when the bottles were plugged with cotton.

THERMAL RELATIONS.—The exact temperature limits for growth of the organism were not determined. Growth occurs, however, from 7° to 35° C. with the optimum at 25° to 28°.

Freshly made transfers on potato-dextrose agar were stored for 5 weeks at a temperature of −25° C. All showed typical growth after 7 days at room temperature.

The thermal death point varied from 55° to 57° C. when fresh nutrient broth cultures in thin-walled tubes were exposed in a water bath for 10 minutes.

VIRULENCE.—The organism has retained its virulence in culture on potato-dextrose agar for over 18 months.

STAINING REACTIONS

Tests made by using Hucker's modification of Gram's stain showed the organism to be Gram positive. Smears from cultures 24 to 96 hours old, growing on potato-dextrose agar and beef-extract agar, were stained between smears of *Phytomonas phaseoli* and *P. flaccumfaciens* (Hedges) Bergey et al. In cultures 6 weeks old several cells stained Gram negative. Also in cultures of this age round bodies (sometimes one, two, or three in a cell) were found which stained darker than the rest of the cell.

The organism was not acidfast. Potato-dextrose agar cultures 48 hours old were treated by Ziehl-Neelsen's method.

TECHNICAL DESCRIPTION

Phytomonas fascians[9] n. sp.

Nonmotile rod 1.5μ to 4.0μ long, 0.5μ to 0.9μ wide, average 2.7μ by 0.69μ. Spores not formed. Gram-positive but not acid-fast. Optimum growth at 25° to 28° C.; thermal death point 55° to 57°. No diastatic action. Gelatin not liquefied but hydrogen sulphide formed. Alkalinity produced in litmus milk and litmus reduced in bottom of tubes; otherwise litmus milk unchanged. Organism aerobic. On potato-dextrose agar cadmium yellow to deep chrome or orange-buff. Nitrates reduced to nitrites. Ammonia produced; no indole formed. Hydrogen-ion concentration increased but no gas found when organism was grown on synthetic carbohydrate medium with following carbon sources: Dextrose, sucrose, maltose, dextrin, glycerol, mannitol, arabinose, xylose, galactose, mannose, and levulose. Gas not found and hydrogen-ion concentration not increased in synthetic carbohydrate medium with lactose, inulin, starch, rhamnose, and raffinose as carbon sources. Slight turbidity with sediment forms in broth and thin fragile pellicle with rim develops on surface. Distinct but very fragile pellicle with rim forms on Fermi's and Uschinsky's solutions; very slight clouding of media. Growth in Cohn's solution not as good as in Fermi's or Uschinsky's. Organism causes development of fasciated growth at base of sweet peas and certain other plants.

DISCUSSION

The fasciation-producing bacterium is another organism that induces cell stimulation in some species of plants. Galls or tumors, such as are caused by *Phytomonas tumefaciens*, *P. beticola* (Smith,

[9] This name was selected in consultation with Prof. F. H. Cowles of the College of Wooster and was first published in a preliminary note in the Fifty-fourth Ann. Rept. Ohio Agr. Expt. Sta. Bull. 561, p. 39, January 1936.

Brown, and Town). Bergey et al., *P. savastanoi* (E. F. S.) Bergey et al., *P. savastanoi* var. *nerii* C. O. Smith, and *P. gypsophilae* (Brown) Bergey et al. are not formed. Stimulation by this organism normally causes numerous fasciated shoots to develop from the base of the stem of the host plant. Apparently, infection does not occur on the roots, and in no instance have any abnormalities resembling those caused by the hairy root organism (*P. rhizogenes* Riker et al.) been observed on diseased plants.

SUMMARY

The cause of fasciation of winter-flowering sweet peas in Ohio has been investigated and found to be a bacterium.

Pathogenic isolates of the organism have been obtained from six different sources. Fifteen single-cell isolates were made from the original cultures and all of these were pathogenic.

Fleshy, fasciated shoots developed at the base of garden peas, petunias, geraniums, tobacco plants, *Gypsophila paniculata* plants, and 10 varieties of winter-flowering sweet peas when they were inoculated with the bacteria. In addition to these hosts, the organism has been isolated from fasciated growths at the base of chrysanthemum plants.

Bacteriological studies are described and the organism is named *Phytomonas fascians* n. sp.

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SPECIFIC EFFECTS OF ZINC APPLICATIONS ON LEAVES AND TWIGS OF ORANGE TREES AFFECTED WITH MOTTLE-LEAF [1]

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INTRODUCTION

This paper presents data on the effects of zinc salts on the growth of leaves and twigs of orange trees affected with mottle-leaf.[2] Trees in an orchard near Riverside, Calif., were selected for observation. They were about 30 years old at the time of treatment and had been severely affected with mottle-leaf for many years. Although the trees had produced a certain amount of new growth each year, the leaves were mottled and dwarfed. Many of the shoots had died back, and the quantity of fruit produced was subnormal.

In March 1934, a short time before the appearance of the first cycle of growth, the trees were sprayed with a mixture containing 10 pounds of zinc sulphate (commercial) and 5 pounds of hydrated lime, in 100 gallons of water. A few weeks after the application of the spray material the trees began to show improvement in the amount and character of the spring growth. This improvement has been maintained during the two following seasons, while untreated trees in adjacent rows have remained unthrifty and have borne typically mottled foliage. Other trees were similarly sprayed in October 1934 and showed normal growth the following spring.

The old, dwarfed leaves on the treated trees did not grow to normal size or shape after treatment, but they produced chloroplasts and assumed the green color of normal leaves. Apparently the leaf cells had lost their meristematic character insofar as multiplication was concerned, but the mitochondria proceeded to develop into plastids which synthesized chlorophyll.[3]

On treated trees it was possible to find in May 1935 many representative twigs bearing the small, elliptic-lanceolate leaves (fig. 1) characteristic of unsprayed mottled trees and also bearing normal green leaves of the 1934 cycles of growth. No such contrasts were observable on the untreated trees. The marked transition from dwarfed to normal leaves was coincident with the production of the first cycle of growth after the application of the spray mixture containing zinc sulphate and lime. This phenomenon indicates that zinc was quickly absorbed by the tree and promptly affected the metabolism of the organs which suffered from the systemic disease mentioned. The application of zinc sulphate to the soil has been

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followed by similar improvements in growth, though the time required for expression has been longer, and injury has resulted in some cases.[4]

OBSERVATIONS ON TWIGS AND LEAVES

The twigs shown in figure 1 were photographed in May 1935. The differences between the leaves produced before and after the
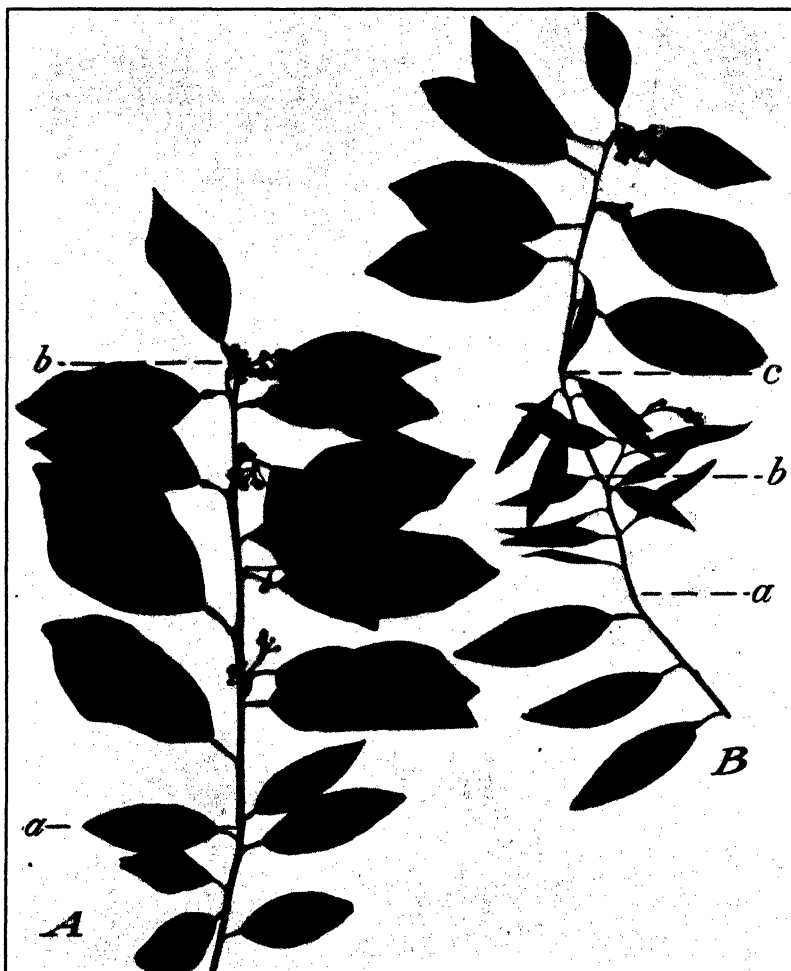
FIGURE 1.—Valencia orange twigs showing the effects of zinc-lime spray on trees affected with mottle-leaf: *A*, Cycle of growth below *a* produced small leaves before the tree was sprayed in October 1934; the cycle *a-b* was produced in the autumn of 1934 and bore large leaves; *B*, *a-b* and *b-c* show the cycles of growth and the small leaves borne before the tree was sprayed; the cycle above *c*, bearing large leaves, was produced after the tree was sprayed in May 1934. Both samples were collected in May 1935.

zinc sulphate-lime mixture was applied are striking. The shoot *a-b-c* in figure 1, *B*, was cut from a tree which had been sprayed in May 1934. During the remainder of the summer it produced one

[4] PARKER, E. R. EXPERIMENTS ON THE TREATMENT OF MOTTLE-LEAF OF CITRUS TREES. Amer. Soc. Hort. Sci. Proc. 31 (sup. vol.): 98–107. 1934.

cycle of growth, starting at the point *c;* this cycle was longer than earlier ones and bore healthy leaves. The greater length of the internodes of the healthy cycle is characteristic of normal growth. The other shoot shown in figure 1, *A*, produced one cycle of growth, *a-b*, after it was sprayed with the zinc sulphate-lime mixture in October 1934. It also bore healthy, oval leaves, which were much larger than those of the preceding cycle. The healthy twigs produced flowers at many of the nodes, in contrast to the few flowers on twigs of the preceding cycles.

The average area of leaves from affected trees gives a more precise indication of the effects of the spray solution containing zinc sulphate. Data were obtained from leaves collected April 15, 1935, from trees which had been sprayed in May 1934, and from comparable unsprayed trees. Each sample consisted of 100 mature leaves selected from the four sides of the trees. The outlines of the leaves were traced on paper and measured with a planimeter. The mean areas of the small leaves on the cycle of growth immediately preceding the treatments were very similar, 0.74 ± 0.01 and 0.75 ± 0.01 square inch for the untreated and for the sprayed trees, respectively. The leaves produced on the late summer or fall cycle of growth were larger on both the control and the treated trees, but the increase in size on the sprayed trees was striking, the figures being 1.61 ± 0.02 and 3.67 ± 0.02 square inches, respectively. The statistical reliability of the means is evident by comparison with their probable errors.

The width of phloem and xylem tissues in twigs may also serve as an index of their growth and hence express the effect of zinc sulphate on cellular activity, at least in respect to the cambium.

On May 7, 1935, twigs were selected from sprayed and unsprayed trees. As in the collection of the leaves, care was taken to obtain representative samples. Only twigs of an ascertainable age were chosen. These were of three ages: (1) Twigs of the 1935 spring cycle; that is, new growth; (2) twigs of the 1934 spring cycle, which had grown approximately 14 months; and (3) twigs of the 1933 spring cycle, which had grown approximately 26 months.

Twenty to thirty twigs were cut to convenient lengths and placed in a 1 : 1 mixture of water and glycerin, where they remained for several weeks. Transverse sections were then cut on a sliding microtome, stained with safranine, washed, and mounted on slides for measurement.

TABLE 1.—*Effect of zinc sulphate-lime spray on the average width of phloem and xylem tissues of orange twigs*

Designations of the twigs (see text)	Average width (microns) of—			
	Phloem in—		Xylem in—	
	Unsprayed twigs	Twigs sprayed May 1934	Unsprayed twigs	Twigs sprayed May 1934
1, 1935 spring cycle (March to May 1935)	127.8±2.3	113.8±2.0	119.6± 5.7	124.3± 3.8
2, 1934 spring cycle (March 1934 to May 1935)	199.7±5.4	187.3±4.9	381.3±14.5	518.5±14.8
3, 1933 spring cycle (March 1933 to May 1935)	249.0±8.0	218.9±5.0	630.8±18.1	716.4±17.3

The data in table 1 show that there was no very significant difference in the thickness of the phloem cylinder in twigs from unsprayed and sprayed trees. The phloem of the older twigs was thicker than that of the younger ones. However, the xylem cylinder was distinctly thicker in the twigs from sprayed than from unsprayed trees. The averages for the young twigs showed no significant difference, but in the case of the two lots of older twigs the differences are statistically significant.

The 26-month-old twigs were nearly a year old before zinc sulphate was applied to the trees on which they grew. Hence, it is not surprising that the difference in size between sprayed and unsprayed twigs in this lot was somewhat less than in the 14-month-old lot.

These measurements may be interpreted to mean that the improvement in general growth activities already described extended to the activity of the cambium and resulted in the production of more wood in these twigs. Although too few data are available to permit a discussion of the specific action of the zinc on the cambium cells, it may be pointed out that the increased cambial activity would logically follow the great increase in chlorophyll-bearing area previously described and the general improvement in the condition of the trees elsewhere reported.[5]

SUMMARY

Orange trees affected with mottle-leaf produced healthy foliage and increased growth after they had been sprayed with a zinc-lime mixture. The specific effects observed were: (1) Larger leaves, (2) longer internodes, and (3) more xylem tissue.

[5] PARKER, E. R. See footnote 4.

THE EFFECT OF LEAF RUST ACCOMPANIED BY HEAT UPON YIELD, KERNEL WEIGHT, BUSHEL WEIGHT, AND PROTEIN CONTENT OF HARD RED SPRING WHEAT [1]

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INTRODUCTION

This paper presents evidence from 4 families from the Ceres × (Hope × Florence) cross comprising a total of 80 selections. These were grown during 2 successive years in experiments to determine yield and other characters. A comparison of these characters for the 2 years shows a marked difference among the families in respect to yield, weight per 1,000 kernels, weight per bushel, and protein content. The weather for the 2 years was similar except that, principally, the July heat in 1935 was decidedly above that in 1934. As the four families differed from one another in resistance to leaf rust and in resistance to flecking, but did not differ greatly in these characters from one season to the other, it is evident that a measure exists by which to determine the injury brought about by flecking and by leaf-rust lesions under conditions of heat well above the optimum for the development of the wheat plant. The results of such measurements are given.

PREVIOUS RESULTS

During the past few years valuable results have been secured by various workers regarding the effect of leaf rust (*Puccinia triticina* Eriks.) upon cereals, particularly with respect to wheat (*Triticum aestivum* L.). It has been recognized from observation that leaf rust upon wheat reduces the yield, and controlled experiments both in the field and greenhouse, such as those conducted by Caldwell et al. (*1*)[2], Mains (*5*), Johnston and Miller (*3*), and others, have given definite information upon this point. Reduction of yield comes about in different ways, but if the plant becomes infected in the earlier stages of its life injury is evident mainly in the formation of fewer and smaller kernels. If the leaf rust attack comes mainly after the blossoming stage then the damage is largely confined to reduced kernel size with a consequent effect upon yield. The effect of leaf rust upon yield per acre and weight of kernel as determined by various individuals is shown in table 1.

Reductions in yield were analyzed by Johnston and Miller, for example, and the yield loss was found to be associated with a decrease in both number and size of kernels. The experimental plants were inoculated at different stages and the greatest losses in yield were found in those groups receiving the earlier inoculations, as might have

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TABLE 1.—*Effect of leaf rust on weight of kernel and yield as secured by various investigators*

Author	Variety	Type	Location	Rust in plant groups		Kernel weight				Loss (−) or gain (+) in yield
				Control	Rusted	Control	Rusted	Loss in weight		
				Percent	*Percent*	*Milligram*	*Milligram*	*Milligram*	*Percent*	*Percent*
Johnston (2)	Malakof	Susceptible	Greenhouse	0	65	30.0	26.0	4.0	13.3	−55.7
	Fulhard	Resistant	do	0	(1)	30.0	29.0	1.0	3.3	−22.3
	Prelude	Susceptible	Field [2]	26	72	21.5	20.9	.6	2.8	
	Turkey	do	do	20	65	21.1	17.4	3.7	17.5	
Mains (5)	Red Fern	do	Greenhouse	0-35	15-85	28.3	21.9	6.4	22.6	−57.2
	Mediterranean	do	do	0-35	10-85	22.9	16.7	6.2	27.1	−63.3
	Fulcaster	do	do	0-5	75-100	35.9	28.9	7.0	19.5	−85.2
	Michigan Amber	do	do	0-1	100	35.8	29.8	6.0	16.8	−33.5
	do	do	do	0-1	75-100	33.0	23.7	9.3	28.2	−37.2
	do	do	do	0-T	90-100	36.6	29.6	7.0	19.1	−27.2
	Illinois No. 1	do	Field	T-35	75-100	30.0	27.5	2.5	8.3	−24.2
Johnston and Miller (3)	Webster	Resistant	Greenhouse	0	(1)	39.6	32.3	7.3	18.4	−11.4
	Pusa	Susceptible	do	0	85	45.0	32.6	12.4	27.6	−75.4
	Warden	Resistant	do	0	17	37.2	33.9	3.3	8.9	−7.0
Caldwell et al. (1)	Shepherd	Susceptible	Field	13	100	33.4	30.9	2.5	7.5	−28.4
	Gladden	do	do	15	100	35.6	31.3	4.3	12.1	−14.8
	Fulhard [3]	do	do	10	100	32.6	30.9	1.7	5.2	+1.2
	Nittany	do	do	10	75	42.7	41.4	1.3	3.0	−8.2
	Purkof	do	do	8	65	32.8	31.2	1.6	4.9	−2.0
	Kawvale	Semiresistant	do	5	45	33.9	32.8	1.1	3.2	+3.4
	Fultz selection	Resistant	do	1	7	33.9				+12.1
Weiss (10)	Marquis	Susceptible	Greenhouse	.5	10	32.4	32.0	.4	1.5	−10.1

[1] Flecked.
[2] Control plants in the field were sulphur-dusted; those in the greenhouse were uninoculated.
[3] Very badly rusted, not resistant.

been expected. A part of the yield reduction was due to decreased weight of kernel, but this reduction in kernel weight did not come about by shriveling, as mainly happens with stem rust (*Puccinia graminis tritici* Eriks. and Henn.), but plumpness was largely retained at the expense of size. This is clearly brought out by the different investigators.

In the experiments cited no mention is made of the relation of temperature to the effects of leaf rust, as this factor did not enter into the experiments summarized in table 1. It is well known that temperature exerts an important influence upon the development of rust, both leaf and stem. Within limits, increasingly high temperatures lessen the time of incubation and result in other modifications, with the usual tendency to increase the virulency of an epidemic. While Lambert (4), Peltier (7), Melander (6), and others have presented observations upon the effect of temperature on the seriousness of stem rust epidemics, but little experimental work appears to have been carried out under controlled conditions of temperature to show the net effect of rust upon the crop in the field under temperatures appreciably divergent. The relation of the rust parasite to its host is complex and the end result is affected by various environmental and internal factors. These have been studied more or less apart from each other, but the integrated effect of such factors upon the host can be determined finally only by empirical methods, particularly under field conditions.

Results secured by the writer with some Ceres × (Hope × Florence) hybrids during the years 1934 and 1935 are of interest in this connection because controlled conditions were rather closely simulated. The hybrids which are of particular interest comprise four families which show different reactions to leaf rust. The reactions to stem rust also differ among the families, but these appear to be of minor importance in this experiment. One of the families, no. 60.4, shows high resistance to leaf rust and even markedly resists flecking. Family no. 54.2 has shown marked resistance to leaf rust at Fargo, North Dakota, but is less resistant to flecking than family 60.4. The two other families, nos. 81.1 and 81.22, are moderately susceptible to leaf rust.

WEATHER IN 1934 AND 1935

The growing seasons of 1934 and 1935 at Fargo were both dryer and hotter than normal growing seasons. In 1934 the excessive heat was in May and July, while in 1935 the high temperatures were limited to July but the heat of that month was excessive. A severe epidemic of stem rust did great damage to the wheat crop in 1935, but evidently it had only a minor effect upon the wheats under discussion as they all showed high resistance. The normal daily mean temperatures for Moorhead, Minn., about 2 miles from the experiment, are given in table 2 in averages of 5-day periods, pentads, and these values are compared with similar average daily means for 1934 and 1935 for the months of May to July. Five-day precipitation totals are also given.

TABLE 2.—*5-day (or 6-day) average daily mean temperatures from May to July 1934 and 1935 compared with similar normal daily mean temperatures and similar data dealing with 5-day precipitation totals* [1]

Period	Temperature					Precipitation		
	Normal	1934	1935	Deviation from normal		Normal	1934	1935
				1934	1935			
	°F.	°F.	°F.	°F.	°F.	Inches	Inches	Inches
May 1-5	49.7	57.8	40.2	8.1	−9.5	0.39	0.79	0.66
May 6-10	51.5	67.8	48.8	16.3	−2.7	.42	.08	.36
May 11-15	54.0	52.2	46.4	−1.8	−7.6	.40	.02	.62
May 16-20	55.9	71.0	58.4	15.1	2.5	.51	.14	0
May 21-25	57.9	53.2	57.6	−4.7	−.3	.46	0	.14
May 26-31	60.0	77.2	57.5	17.2	−2.5	.57	.12	.03
Total						2.75	1.15	1.81
June 1-5	61.7	68.4	52.6	6.7	−9.1	.51	.73	.36
June 6-10	62.5	59.4	58.0	−3.1	−4.5	.60	1.42	.17
June 11-15	63.9	60.8	66.4	−3.1	2.5	.68	0	.95
June 16-20	65.8	66.4	61.8	.6	−4.0	.62	1.05	0
June 21-25	66.1	69.6	64.4	3.5	−1.7	.64	.70	1.53
June 26-30	67.3	72.0	68.4	4.7	1.1	.78	.03	0
Total						3.83	3.93	3.01
July 1-5	67.8	68.0	76.8	.2	9.0	.74	.28	.01
July 6-10	68.5	68.4	70.4	−.1	1.9	.48	.04	.83
July 11-15	68.9	72.2	72.4	3.3	3.5	.57	.36	.74
July 16-20	69.3	78.8	77.8	9.5	8.5	.59	0	.04
July 21-25	69.0	80.0	77.0	11.0	8.0	.38	.10	.12
July 26-31	68.5	68.7	78.0	.2	9.5	.53	.02	.39
Total						3.29	.80	2.13
Average or total	62.7	67.3	62.9	4.6	.3	9.87	5.88	6.95

[1] Data are from the official records of the U. S. Weather Bureau.

During May 1934 there were three 5-day periods of excessive heat with one other well above the average. Rains meanwhile were deficient. Both May and June, 1935, were definitely below normal in temperature, the daily deficiency averaging 3°. June temperatures for 1934 were 1.6° above normal. July in 1934 and again in 1935 had temperatures decidedly above normal; these averaged +4° daily for 1934 and +6.7° for 1935. The absolute maxima for July for the 2 years were 106° and 96° F., respectively. The 3 months in 1935 showed a decidedly lower daily average temperature than did the same months in 1934. The belief is common that in 1935 the small-seeded cereals, especially wheat, suffered more from excessive July heat than they did in 1934. There is evidence that this belief is founded in fact, as will appear later, yet the daily excess temperature for July 1935 was but 2.7° above the daily average for July 1934. Also, the crop in 1934 received decidedly more heat during its post-heading period than was the case in 1935. It has been suggested that the marked damage in 1935 may have been associated with the profound change from the very cool weather of May and June to the excessive heat of July, and the inability of the plants to adapt themselves to the radically new environment. While this conclusion is rather speculative it is well to give it consideration. The presence of the stem rust epidemic makes it difficult to evaluate the effect of the heat alone on most wheat varieties.

EXPERIMENTAL PROCEDURE

In 1934, 24 selections were taken of each of the 4 families mentioned above and 1 other family [3] and grouped into 6 experimental units each of which carried 4 hybrid selections from each family plus a check variety grown four times or 2 checks each grown 2 times. Each experimental unit thus contained 24 wheats from which yields were to be obtained. In each experimental unit each selection or check was triplicated and the rod rows were arranged in a random manner. In 1935 one of the six experimental units was located some distance from the other five on a different soil type. Because the yield of this unit was decidedly below that of the rest of the experiment it was omitted, as was the corresponding experiment of 1934. This left 5 experimental units for each year, each unit of which contained 20 of the hybrids plus 4 checks. In this paper, for purposes of comparison, the one family indicated and the checks are omitted. Thus there are 80 of the Ceres × (Hope × Florence) hybrid selections for comparison grouped into 4 families grown in duplicate during the 2 years, plus the 4 checks for each experimental unit. There was a slight change in the checks from the one year to the next, and in 1935, contrary to the practice in 1934, the yield rows were guarded. A study of the statistical constants of the two experiments shows that the absence of the guard rows in 1934 does not invalidate the comparisons which are made here.

EXPERIMENTAL RESULTS

YIELD AND WEIGHT DATA

The detailed data with respect to yield, weight per 1,000 kernels, and weight per bushel of the selections of the four families for the 2 years, and the respective leaf rust readings are shown in table 3.

The data for each family of selections are arranged together even though different experimental units are concerned, as all units were given comparable treatment. While yields are distinctly lower for 1935 for three of the families they are essentially the same for family 60.4. In this family for 1935 a trace of leaf rust is reported in each case, but these readings are probably too conservative and a zero reading would have been more nearly correct. Plants showing even minute pustules were unusual. No more than a trace of stem rust was observed in 1934.

The results can be better appreciated when summarized as in table 4.

[3] This other family, no. 81.45, is omitted as it is believed that its yields in 1934 were relatively low because of a thin stand, which may have resulted in part from thin seeding due to very large kernels. It is not evident the omission of this family prejudices the study in any way.

TABLE 3.—*Data on 4 families of Ceres × (Hope × Florence wheat) with respect to leaf and stem rust, yield, kernel weight, and weight per bushel for 1934 and 1935*

Experiment no.	Family and selection no.	Leaf rust		Stem rust, 1935	Yield per acre			Weight per 1,000 kernels			Weight per bushel		
		1934	1935		1934	1935	Difference	1934	1935	Difference	1934	1935	Difference
	Family 54.2:	Pct.	Pct.	Pct.	Bu.	Bu.	Bu.	G.	G.	G.	Lb.	Lb.	Lb.
1	1	1	1	2	38.3	35.2	-3.1	33.1	27.1	-6.0	59.0	56.0	-3.0
2	3	1	3	1	35.7	28.3	-7.4	33.1	26.6	-6.5	59.5	54.5	-5.0
3	5	1	1	2	37.5	32.8	-4.7	32.4	27.5	-4.9	57.5	56.0	-1.5
4	6	1	1	3	38.8	27.7	-11.1	33.3	27.2	-6.1	59.5	55.0	-4.5
5	7	T	1	2	40.5	24.6	-15.9	30.2	24.7	-5.5	59.0	55.0	-4.0
6	8	T	1	4	41.9	30.1	-11.8	34.0	27.3	-6.7	59.0	55.0	-4.0
7	9	T	2	3	39.4	30.4	-9.0	32.4	29.0	-3.4	58.5	54.0	-4.5
8	10	T	1	3	37.5	27.7	-9.8	31.2	27.7	-3.5	58.5	55.5	-3.0
9	11	T	4	3	35.7	35.0	-.7	31.6	28.3	-3.3	58.0	55.5	-2.5
10	14	T	1	2	37.6	30.4	-7.2	30.3	27.0	-3.3	58.5	55.5	-3.0
11	15	T	1	2	35.3	32.3	-3.0	31.7	26.6	-5.1	58.0	54.5	-3.5
12	16	1	1	1	38.9	29.1	-9.8	30.7	26.3	-4.4	58.0	55.0	-3.0
13	17	T	1	2	36.1	32.6	-3.5	31.4	27.2	-4.2	58.0	55.0	-3.0
14	18	T	T	2	36.9	31.9	-5.0	31.3	24.4	-6.9	58.0	56.0	-2.0
15	19	T	1	2	37.1	31.8	-5.3	30.5	26.6	-3.9	58.5	55.5	-3.0
16	21	T	T	3	37.7	34.1	-3.6	32.3	28.2	-4.1	58.5	56.0	-2.5
17	22	3	5	1	29.8	31.6	+1.8	31.9	24.7	-7.2	58.0	55.0	-3.0
18	23	1	1	2	34.4	31.8	-2.6	32.9	25.8	-7.1	58.5	55.0	-3.5
19	24	1	2	1	34.7	29.6	-5.1	28.9	23.5	-5.4	57.5	53.5	-4.0
20	25	2	2	1	34.2	32.5	-1.7	32.4	26.5	-5.9	58.0	54.5	-3.5
	Average	0.6	1.5	2.2	36.9	31.0	-5.9	31.8	26.6	-5.2	58.4	55.1	-3.3
	Family 60.4:												
1	1	T	T	0	35.4	32.8	-2.6	34.5	31.6	-2.9	59.0	56.5	-2.5
2	2	T	T	1	38.3	33.2	-5.1	35.1	30.8	-4.3	59.5	56.0	-3.5
3	3	T	T	T	33.5	39.5	+6.0	35.2	30.8	-4.4	60.0	58.5	-1.5
4	4	T	T	2	35.6	35.7	+.1	34.5	30.7	-3.8	59.5	56.0	-3.5
5	5	T	T	2	41.5	32.0	-9.5	33.0	26.3	-6.7	59.5	54.5	-5.0
6	6	1	T	3	32.3	34.3	+2.0	34.1	30.2	-3.9	58.5	56.5	-2.0
7	7	T	T	2	35.2	37.2	+2.0	34.7	32.6	-2.1	59.0	56.5	-2.5
8	8	T	T	5	43.8	34.0	-9.8	34.5	29.3	-5.2	59.0	55.5	-3.5
9	9	T	T	2	36.5	34.5	-2.0	33.1	31.1	-2.0	59.0	57.0	-2.0
10	10	T	T	T	32.0	33.2	+1.2	31.5	27.4	-4.1	58.5	56.5	-2.0
11	11	T	T	T	35.1	35.6	+.5	32.5	31.4	-1.1	58.5	58.0	-.5
12	12	T	T	T	35.7	34.5	-1.2	30.7	32.3	+1.6	59.0	57.5	-1.5
13	13	T	T	2	32.8	32.8	0	32.2	31.0	-1.2	59.0	58.0	-1.0
14	14	T	T	1	32.8	35.8	+3.0	31.5	29.4	-2.1	58.5	56.5	-2.0
15	15	T	T	3	34.3	36.5	+2.2	32.8	29.8	-3.0	59.0	58.0	-1.0
16	16	T	T	2	36.6	37.1	+.5	31.1	31.6	+.5	59.0	57.5	-1.5
17	17	T	T	3	28.7	36.5	+7.8	32.8	33.0	+.2	57.5	57.5	0
18	18	1	T	1	27.8	36.1	+8.3	32.0	28.9	-3.1	58.0	56.5	-1.5
19	19	T	T	T	34.8	40.8	+6.0	32.8	32.3	-.5	57.5	56.5	-1.0
20	20	T	T	2	32.9	34.8	+1.9	31.5	26.7	-4.8	58.5	56.0	-2.5
	Average	T	T	1.6	34.8	35.5	+0.7	33.0	30.4	-2.6	58.8	56.8	-2.0
	Family 81:1:												
1	1	2	3	T	37.8	31.8	-6.0	38.3	29.6	-8.7	60.0	56.0	-4.0
2	2	2	4	T	38.4	30.1	-8.3	35.2	30.6	-4.6	60.0	57.5	-2.5
3	3	11	23	T	37.7	28.0	-9.7	37.6	24.6	-13.0	59.5	53.5	-6.0
4	5	7	10	1	37.7	29.2	-8.5	37.8	25.7	-12.1	60.0	54.0	-6.0
5	6	11	22	T	37.5	24.6	-12.9	37.1	25.7	-11.4	58.5	52.5	-6.0
6	7	11	18	T	37.3	26.1	-11.2	35.9	30.5	-5.4	59.5	54.0	-5.5
7	8	11	17	T	36.9	27.1	-9.8	33.8	22.3	-11.5	59.5	53.5	-6.0
8	9	8	13	T	34.6	27.9	-6.7	36.0	31.8	-4.2	59.5	54.0	-5.5
9	10	5	7	T	35.9	30.9	-5.0	35.2	28.1	-7.1	58.0	55.0	-3.0
10	11	3	10	T	36.1	31.8	-4.3	33.5	28.2	-5.3	59.0	55.5	-3.5
11	12	4	10	T	33.4	30.1	-3.3	34.2	26.3	-7.9	59.0	56.0	-3.0
12	13	2	11	T	34.5	30.3	-4.2	34.5	26.6	-7.9	58.5	55.0	-3.5
13	14	6	7	T	35.4	23.2	-12.2	33.4	22.5	-10.9	59.0	54.5	-4.5
14	15	5	6	T	36.4	27.2	-9.2	33.1	24.0	-9.1	59.0	55.0	-4.0
15	16	2	5	T	36.5	33.5	-3.0	35.0	28.6	-6.4	58.5	57.0	-1.5
16	17	7	22	T	33.0	25.4	-7.6	32.6	25.4	-7.2	58.5	55.0	-3.5
17	18	6	13	T	33.1	27.8	-5.3	32.9	26.2	-6.7	59.0	54.5	-4.5
18	19	4	16	T	33.0	29.8	-3.2	32.1	22.8	-9.3	59.0	55.0	-4.0
19	20	3	6	T	32.4	29.6	-2.8	33.8	25.6	-8.2	58.0	54.5	-3.5
20	21	4	23	T	30.6	26.6	-4.0	37.1	25.6	-11.5	58.5	53.5	-5.0
	Average	3.7	12.3	T	35.4	28.5	-6.9	35.0	26.5	-8.5	59.0	54.8	-4.2

TABLE 3.—*Data on 4 families of Ceres × (Hope × Florence wheat) with respect to leaf and stem rust, yield, kernel weight, and weight per bushel for 1934 and 1935*—Continued

Experiment no.	Family and selection no.	Leaf rust 1934	Leaf rust 1935	Stem rust, 1935	Yield per acre 1934	Yield per acre 1935	Yield per acre Difference	Weight per 1,000 kernels 1934	Weight per 1,000 kernels 1935	Weight per 1,000 kernels Difference	Weight per bushel 1934	Weight per bushel 1935	Weight per bushel Difference
	Family 81.22:	*Pct.*	*Pct.*	*Pct.*	*Bu.*	*Bu.*	*Bu.*	*G.*	*G.*	*G.*	*Lb.*	*Lb.*	*Lb.*
1	1	6	15	T	35.1	25.0	−10.1	35.5	26.2	−9.3	60.5	56.0	−4.5
2	2	11	13	T	37.8	25.0	−12.8	37.4	23.2	−14.2	61.0	56.5	−4.5
3	3	8	18	T	37.1	29.0	−8.1	33.6	24.5	−9.1	61.0	56.5	−4.5
4	4	9	15	T	38.2	28.0	−10.2	34.6	23.3	−11.3	61.0	54.5	−6.5
5	5	8	12	1	39.9	27.1	−12.8	33.9	25.4	−8.5	61.0	56.0	−5.0
6	7	13	13	3	40.5	26.6	−13.9	33.2	23.6	−9.6	61.0	56.0	−5.0
7	8	5	9	1	41.3	24.6	−16.7	34.3	22.8	−11.5	60.5	55.5	−5.0
8	9	6	9	T	41.7	30.0	−11.7	35.7	25.1	−10.6	60.5	56.0	−4.5
9	10	5	8	T	36.9	26.2	−10.7	33.1	24.2	−8.9	60.0	55.5	−4.5
10	12	4	12	T	37.3	28.4	−8.9	32.5	24.8	−7.7	60.0	56.5	−3.5
11	13	4	7	T	37.0	27.2	−9.8	31.5	23.3	−8.2	60.0	57.0	−3.0
12	14	5	11	T	35.2	21.5	−13.7	33.2	23.3	−9.9	59.5	53.5	−6.0
13	15	5	15	T	37.1	27.6	−9.5	32.4	23.7	−8.7	60.0	55.0	−5.0
14	16	8	18	T	36.7	26.7	−10.0	33.3	25.0	−8.3	59.5	54.5	−5.0
15	17	5	12	T	37.0	27.5	−9.5	32.6	24.0	−8.6	60.5	56.5	−4.0
16	19	8	15	1	40.1	27.1	−13.0	32.9	23.9	−9.0	59.5	54.5	−5.0
17	20	7	15	T	36.8	27.1	−9.7	32.2	22.2	−10.0	60.0	53.5	−6.5
18	21	7	18	T	33.0	29.5	−3.5	31.7	22.1	−9.6	60.0	55.0	−5.0
19	22	6	15	T	33.5	28.8	−4.7	31.9	22.1	−9.8	60.0	55.5	−4.5
20	23	10	15	T	35.0	25.3	−9.7	30.6	21.7	−8.9	60.0	54.0	−6.0
	Average	7.0	13.2	0.3	37.4	26.9	−10.5	33.3	23.7	−9.6	60.3	55.4	−4.9

TABLE 4.—*Quantitative changes summarized from table 3 in some Ceres × (Hope× Florence) hybrid families from 1934 to 1935*

YIELD PER ACRE

Family	1934 Leaf rust [1]	1934 Mean	1934 Standard error Value [2]	1934 Standard error Percent [3]	1935 Rust Leaf	1935 Rust Stem	1935 Mean	1935 Standard error Value [2]	1935 Standard error Percent [3]	Increase or decrease in 1935 Bushels	Increase or decrease in 1935 Percent
	Percent	*Bushels*	*Value*	*Percent*	*Percent*	*Percent*	*Bushels*	*Value*	*Percent*	*Bushels*	*Percent*
60.4	Trace	34.8	0.83	10.36	Trace	1.6	35.5	0.50	6.16	0.7	2
54.2	0.6	36.9	.59	6.92	1.5	2.0	31.0	.59	8.31	−5.9	−16
81.1	5.7	35.4	.49	6.09	12.3	Trace	28.5	.59	8.99	−6.9	−19
81.22	7.0	37.4	.54	6.26	13.2	.3	26.9	.44	7.14	−10.5	−28

WEIGHT PER 1,000 KERNELS

Family		Mean	Standard error Value	Standard error Percent			Mean	Standard error Value	Standard error Percent	Bushels	Percent
		Grams					*Grams*				
60.4		33.0	0.32	4.16			30.4	0.42	6.08	−2.6	−8
54.2		31.8	.28	3.84			26.6	.31	5.13	−5.2	−16
81.1		35.0	.42	5.23			26.5	.61	10.05	−8.5	−24
81.22		33.3	.36	4.70			23.7	.27	4.98	−9.6	−29

WEIGHT PER BUSHEL

Family		Mean	Standard error Value	Standard error Percent			Mean	Standard error Value	Standard error Percent	Pounds	Percent
		Pounds					*Pounds*				
60.4		58.8	0.14	1.06			56.8	0.22	1.66	−2.0	−3
54.2		58.4	.13	.96			55.1	.15	1.20	−3.3	−6
81.1		59.0	.14	1.02			54.8	.27	2.17	−4.2	−7
81.22		60.3	.12	.85			55.4	.23	1.84	−4.9	−8

[1] Stem rust essentially zero in 1934.   [2] Standard error for family mean.   [3] Equals variability.

LEAF RUST READINGS

An attempt was made to read a pustuled area with a reasonable degree of accuracy. A leaf, dried, was taken from one of the selections of family 81.22 and a portion measuring 11 by 20 mm was marked off into 20 squares and viewed with a low-power compound microscope. The total area was sampled, once for each square, and the area of each pustule in each field measured approximately by a micrometer scale. The 20 fields covered 76.03 mm^2 of leaf surface, of which 5.55 mm^2, or 7.3 percent, was found to be pustular. As these pustules had passed into the teliospore stage their outlines were fairly definite but in their subepidermal condition the edges were indistinct. According to the standard adopted by the Division of Cereal Crops and Diseases, United States Department of Agriculture, and commonly used in rust readings, 7.3 percent of the area covered would be equal to a rust reading of 20 percent. This particular selection had a leaf-rust reading estimated in the field as 13 percent. Accurate reading upon a single leaf blade might easily show much divergence from an estimated average for the selection. However, the pustules observed under the microscope were small. Nearly one-third of the estimated total of 446 found on the 220 mm^2 had an average area of but 0.013 mm^2, equal to a circle diameter of 0.13 mm. Pustules of such small size would escape attention in ordinary rust readings. A microscopic examination of leaves from family 60.4 failed to show any pustules, although certain of the relatively few minute yellowed areas were probably flecks. Pustules on leaves of family 54.2 could be found, although they were far less common and of smaller size than on leaves of family 81.22. Flecking on these leaves was markedly greater than on leaves of family 60.4 but less than on those of family 81.22.

COMPARISON OF 1934 AND 1935 RESULTS

It is evident that the work described above was not carried out under controlled conditions in the usual sense of the term. One variety is not subject to the presence and absence of leaf-rust infection under two controlled conditions of temperature. In lieu of this the two temperature gradients are supplied by growing identical crops under similar conditions for 2 successive years which differ materially, and probably mainly, in the amount and rate of heat delivered to the crop during the time required for the disease to develop. In place of one variety divided in such a manner that a portion becomes rusted, we have here closely related families which differ in their reactions to rust.

The results for 1934 show that the four families behaved similarly as to yield and other indicated characters. Any disparities in 1934 may be explained in part by accidental conditions, such as a slight shattering in family 60.4. The same wheats showed a very different behavior in 1935. The question then arises: Is this difference due to the combined effect of leaf rust and heat in 1935 or would the disparities of 1935 have appeared had there been the increased heat without the leaf rust? While opinion may differ, the evidence indicates that leaf rust, associated with high temperatures, was the responsible agent. In short, the results presented herewith may be considered essentially equivalent to those of a controlled experiment.

The crop was subjected to the entire weather picture, and obviously there are fewer gaps to bridge when conclusions are extended to field results than if the extension were to be made from greenhouse experiments.

Stem rust was low in 1935 for all families, the maximum average reading being 2.2 percent for family 54.2. Injury resulting from an amount of rust so low as this is rather minor. Most of the rust was located near the nodes and thus translocation was not much disturbed. The families are placed in ascending order of leaf-rust reading and the 1935 yields fall into a contrary order. The yield of family 60.4 was significantly below that of family 81.22 in 1934. This was partly due to the fact that there was a little shattering of 60.4 in 1934. Moreover, this family was cut somewhat earlier in 1934 because of its tendency to shatter. Judging from the decrease of 8 percent in weight per 1,000 kernels and of 3 percent in weight per bushel of family 60.4 from 1934 to 1935, the yield of this family probably suffered in 1935 because of high temperatures. The 1934 yield also probably suffered because of the reduction mentioned from the optimum otherwise obtainable.

The difference in leaf rust in 1935 of 1.5 percent between families 60.4 and 54.2 is apparently too small to influence yield as a direct result of the parasite. As the weight per 1,000 kernels and weight per bushel are both lower for family 54.2, the major portion of the difference in yield of 4.5 bushels is pretty certainly due to seasonal effect. In 1934 family 60.4 yielded 2.1 bushels less than family 54.2. The major factor which brought about a relative reduction of yield in family 54.2 as compared to family 60.4, in 1935, is believed to have been the deterioration of the leaf due to the flecking caused by leaf rust. This premature yellowing of the leaf before the normal ripening of the plant, which was probably followed by necrosis and which interfered with normal photosynthesis, naturally affected seed production. With the greater amount of leaf rust in the other two families, 81.1 and 81.22, the deterioration of the leaf was carried still farther and the effect upon the crop was still greater. In the present instance it does not seem possible to distinguish between the injury brought about incidentally by the parasite as a result of flecking, commonly followed by necrosis, and the direct effect of the parasite, in the utilization of food of the host. A study of the mature plants of families 81.1 and 81.22 leads to the belief that the latter family was subject to greater infection in 1935, although table 3 shows that infection upon the leaf blade, used in making the readings, was nearly the same in the two cases. Family 81.22 appeared to carry more leaf-rust pustules upon the sheaths and upon the peduncles, but it is not easy to obtain quantitative evidence on this point. If a real difference existed in this respect we would probably find here an explanation of the much smaller yield of family 81.22 as compared with family 81.1. One cannot be certain that the pathogen continued to act injuriously upon the host until the latter was nearly or quite mature but this seems a reasonable supposition.

The yields and weights per 1,000 kernels for 1935 reduced to percentages with family 60.4 as the base are shown in table 5.

Families 54.2 and 81.22 show almost exact proportional deficiency in yield and weight of kernel when compared with family 60.4. Evi-

dently differences in yield are attributable mainly to those factors which brought about a smaller kernel. The difference between families 81.1 and 60.4, in respect to yield is perhaps significantly greater than in respect to kernel weight.

The weights per bushel show much smaller differences among each other than the corresponding ones for yield and kernel weight. Family 60.4 has the highest bushel weight, 56.8 pounds, which is 2 pounds, or 3 percent, lower than its bushel weight for 1934. This is probably a fair indication of the loss that resulted from the excess heat of 1935. The lower yielding families of 1935 had bushel weights lower than that of family 60.4. While the maximum differences with family 81.1, was but 2 pounds, or 3.5 percent, statistically this is strikingly significant. The odds are several million to one against the probability that a mean deviation as large as 2 pounds could accidentally arise.

TABLE 5.—*Yields and weights per 1,000 kernels reduced to percentages with the results for family 60.4 as a base, 1935*

Family	Yield	Weight of 1,000 kernels	Family	Yield	Weight of 1,000 kernels
	Percent	*Percent*		*Percent*	*Percent*
60.4	100	100	81.1	80	87
54.2	88	87	81.22	76	78

Few results are found in the literature concerning the effect of leaf rust on bushel weight. Caldwell et al. (*1*) secured bushel weights on winter wheat grown outside which had been sulphur-dusted to control leaf rust. Omitting two varieties which showed reversal of yields, perhaps due to sulphur injury, they found that the weight of the grain produced by the five varieties on the control plots was 59.9 pounds per bushel, while the bushel weight from the corresponding rusted plots was 59.0 pounds. The corresponding loss in yield of grain averaged 11 percent. The leaf-rust readings averaged 90 and 11 percent, respectively, for the rusted and control plots. While the rust readings in the experiments cited were much higher than in the present work, the falling off in yields from family 60.4 to the average of the three other families is 18 percent as compared to 11 percent in the winter wheat experiment. The change in bushel weight of 1.7 pounds, or 3 percent, from family 60.4 to the average of the three other families, is perhaps comparable to that secured by Caldwell and his associates.

A measure of injury resulting from a small amount of stem rust is obtainable from the present data. Family 60.4 contains 14 selections which averaged 2 percent of stem rust in 1935 while the other 6 carried no more than a trace. For convenience of study this family is divided into two groups, and the values are shown in table 6.

The group carrying 2 percent of stem rust in 1935 yielded 0.5 bushel more in 1934 and 1.1 bushels less in 1935 than the group carrying a trace of stem rust, a total difference of 1.6 bushels. Similarly, the kernel weight was 0.2 g higher in 1934 and 0.9 g lower in 1935, making a total difference of 1.1 g. The bushel weights were equal in 1934, but in the 1935 group with 2 percent rust weighed 0.7 pound less per bushel. These differences are small, but as they in-

TABLE 6.—*Separation of family 60.4 into two groups characterized by a trace and by 2 percent of stem rust in 1935*

Selection nos.	Rust			Yield per acre				Weight per 1,000 kernels				Weight per bushel			
	Leaf		Stem	1934	1935	Differ-ence		1934	1935	Differ-ence		1934	1935	Differ-ence	
	1934	1935	1935												
	Pct.	*Pct.*	*Pct.*	*Bu.*	*Bu.*	*Bu.*	*Pct.*	*Grams*	*Grams*	*Grams*	*Pct.*	*Lbs.*	*Lbs.*	*Lbs.*	*Pct.*
9.60.4.1,3, 10–12, 19.	T	T	T	34.4	36.1	+1.7	+5	32.9	31.0	−1.9	−6	58.8	57.3	−1.5	−3
9.60.4.2, 4–9, 13–18, 20	T	T	2	34.9	35.0	+0.1	0	33.1	30.1	−3.0	−9	58.8	56.6	−2.2	−4

volve three characters they probably reflect the action of the parasite. No doubt under ordinary circumstances the effect of so little stem rust could not be detected in the plant, but with the high temperatures accompanying the 1935 epidemic this evidently became possible.

EFFECT OF HEAVY STEM RUST WITH LOW TEMPERATURES

It is a commonplace that if stem rust injures the wheat crop evidence of such injury appears in reduced yields and in shrunken kernels leading to reduced weights per bushel. However, a high bushel weight may accompany a rather high rust reading. In 1927 data were secured from the wheat nursery at Fargo which illustrate this point and are of particular interest here as they serve to throw light on the effect of rust on yield, and on bushel weight in this case, when accompanied by differences in temperature. The data are shown in table 7.

TABLE 7.—*Effect of leaf and stem rust on yield and bushel weight of various wheat varieties under cool temperature conditions, 1927*

Variety	Date of harvest	Rust		Yield per acre	Bushel weight
		Leaf	Stem		
		Percent	*Percent*	*Bushels*	*Pounds*
Marquis	Aug. 13	2	53	34.7	61.5
Ceres	Aug. 10	4	3	33.3	60.4
Axminster	Aug. 12	3	30	32.5	61.5
Reliance	Aug. 13	5	68	30.1	59.5
Renfrew	Aug. 14	2	75	22.7	53.0

Except for Ceres the stem rust readings on these wheats were high. Bushel weight and yield for Marquis and Axminster were not appreciably lowered as compared with those for Ceres. Evidently Reliance was somewhat adversely affected, but only Renfrew, a late and very susceptible variety, was seriously injured. In 1935 Marquis and Canus (the latter equivalent to Reliance) were grown in the nursery, and with rust readings at 65 and 75 percent, respectively, the respective yields were 7.7 and 6.8 bushels per acre and the respective bushel weights were 38.5 and 36.0 pounds.

The season of 1927 was cool, which caused the harvest to be nearly 2 weeks later than that of 1935. The average daily mean temperature from July 1 to August 13 was 2.8° F. below the normal daily mean, and only during the last 3 days of this period did the daily mean rise appreciably above the normal. This period of heat probably came too late to cause any marked rust injury. In 1935 the average daily mean temperature for July was 6.8° F. above the normal daily mean. Thus during the post-heading period for the two crops the difference of the two average daily means showed a daily disparity of nearly 10° F. Evidently, temperature exerts a very pronounced influence upon the severity of stem rust. An increase of heat rapidly increases the injury caused by the parasite. The same is evidently true of leaf rust. Injury from leaf rust may result directly from the action of the parasite or, indirectly, from the flecking produced by spore invasion which brings about a loss of chlorophyll tissue and often leads to extensive leaf yellowing and necrosis. In either case added heat results in increased loss.

RELATION OF LEAF RUST TO PROTEIN CONTENT

Caldwell et al. (*1*), in their studies of the effect of leaf rust upon the composition of winter wheat, found that the protein content was much affected, particularly so far as susceptible varieties were concerned. With increasing intensity of leaf rust the protein content in the grain decreased. In one instance the protein percentages were 11.5, 11.0, 10.5, and 10.2, as leaf-rust infection increased, either in amount of infection or in length of time infected. In the present study protein determinations [4] were made for 1934 and 1935, and the values secured are shown in table 8.

The first four protein determinations of family 54.2 for 1934 are decidedly below the other values in the column. The same is true for the corresponding top two values of family 60.4, the top four of family 81.1, and the top three of 81.22. These values are for wheats grown in a single experiment, and the reason for their unusual behavior with regard to protein is not clear. So far as known growth conditions were comparable for all of the selections grown in 1934. Comparisons were made with the values as they appear in the table and also when the 13 values indicated, with the corresponding 1935 values, are omitted. A study of the differences in protein content for the 2 years should show the effects of different amounts of leaf rust under different temperature conditions upon this character. The results are summarized in table 9.

TABLE 8.—*Protein content, with differences, of four families of Ceres × (Hope × Florence) wheats grown comparably in 1934 and in 1935*

Family and selection no.	1934	1935	Difference	Family and selection no.	1934	1935	Difference
Family 54.2:				Family 81.1:			
1	13.8	16.4	2.6	1	13.5	16.0	2.5
3	14.1	16.7	2.6	2	14.4	15.9	1.5
5	13.7	16.2	2.5	3	13.8	1.2	1.4
6	13.7	15.9	2.2	5	14.0	16.2	2.2
9	15.1	16.5	1.4	6	15.7	15.1	—.6
10	15.2	16.0	.8	7	16.5	15.6	—.9
11	15.8	16.1	.3	8	15.9	15.2	—.7
14	15.7	16.1	.4	9	15.8	15.7	—.1
15	15.7	16.2	.5	10	16.3	15.5	—.8
17	16.0	16.5	.5	11	16.2	15.0	—1.2
18	15.7	16.0	.3	12	16.5	15.5	—1.0
19	15.9	16.5	.6	13	16.3	15.6	—.7
21	15.4	15.9	.5	14	16.0	15.0	—1.0
22	16.5	15.6	—.9	15	16.4	15.1	—1.3
23	15.9	15.8	—.1	16	16.2	15.3	—.9
24	15.7	16.2	.5	17	16.4	15.1	—1.3
25	15.9	16.0	.1	18	16.7	15.7	—1.0
				19	16.5	15.8	—.7
Average	15.3	16.2	.87	20	16.5	16.0	—.5
				Average	15.8	15.5	—.27
Family 60.4:							
2	13.7	15.5	1.8	Family 81.22:			
4	13.7	16.3	2.6	1	13.1	15.1	2.3
6	15.5	15.7	.2	3	13.9	15.3	1.1
7	15.5	16.0	.5	4	12.8	15.1	2.3
9	15.2	15.5	.3	5	15.7	15.8	.4
11	15.7	15.6	—.1	7	15.8	15.5	—.4
13	15.2	16.0	.8	8	15.5	15.9	.2
14	15.5	15.5	0	9	15.9	15.7	—.2
15	15.4	15.9	.5	10	16.3	16.1	—.5
16	15.3	15.6	.3	12	16.5	16.0	—.4
17	16.0	15.8	—.2	14	15.6	16.0	.
18	15.6	15.7	.1	15	15.8	15.8	0
19	15.6	15.5	—.1	17	16.2	15.8	—.4
				19	15.8	15.9	.1
Average	15.2	15.7	.52	20	15.7	15.7	0
				21	16.6	15.9	—.7
				23	15.6	15.7	.1
				Average	15.4	15.7	.28

TABLE 9.—*Values calculated from data in table 8 to show differential effects of leaf rust upon differences of protein content in certain wheat families in 2 years*

Data used	Item		Values for family			
			54.2	60.4	81.1	81.22
Using all pairs	Plant selections (per family) .... number		17	13	19	16
	Mean .... percent		0.87	0.52	—0.27	0.28
	Standard deviation		1.00	0.78	1.17	0.84
	Z value		0.87	0.67	0.23	0.33
	Odds		620:1	43:1	5:1	8:1
Omitting series 329 and 368 for 1934 and 1935, respectively	Plant selections (per family) ....number		13	11	15	13
	Mean .... percent		0.38	0.21	—0.85	—0.09
	Standard deviation		0.50	0.29	0.31	0.33
	Z value		0.75	0.71	2.76	0.28
	Odds		81:1	40:1	+10,000:1	4:1

The two families least injured by leaf rust had a larger protein content, which was of significant value, in 1935 than in 1934. Family 60.4, which showed least leaf deterioration, had a smaller increase than family 54.2. The two families showing greatest damage from leaf rust and greater relative damage in 1935, as compared with the two other families, showed either a decrease of protein content in 1935

or an increase that was not significant. Family 81.22, having the greatest relative reduction in yield and weight per 1,000 kernels in 1935, showed a smaller decrease in protein content in the second year than family 81.1. If families 54.2 and 60.4 are compared as a group with families 81.1 and 81.22 the difference in protein content for the two groups for the 2 years is 0.73 ± 0.17 percent. The odds are thus 370:1 that the wheats showing least injury from leaf rust in 1935 were less affected in the amount of protein laid down in the grain than were other wheats showing greater injury from leaf rust in 1935 as compared with 1934. The leaf-rust readings in families 81.1 and 81.22 were nearly the same for the 2 years, and in both years the readings were moderate. The high temperatures of 1935 in conjunction with the leaf rust present reduced the protein content measurably, but apparently yield, weight per 1,000 kernels, and weight per bushel were more susceptible to the effects of leaf rust than was protein content.

DISCUSSION

Evidence has been presented indicating that a necrotic condition of the wheat leaf induced by leaf rust may influence yield and other characters very decidedly in a season such as 1935 when temperatures are high. The yield deficiency is shown to be aggravated when the necrotic areas are accompanied by leaf rust lesions. When these lesions are present leaf deterioration increases and the pustular effects afford another probable source of injury. Weaver (*9*) describes an experiment conducted by Humphrey with young wheat plants affected by leaf rust in which transpiration was increased 38 percent when less than 1 percent of the transpiration area of the wheat plant was involved by the rust pustules. The area covered by leaf rust in families 81.1 and 81.22 was evidently larger than on the plants studied by Humphrey. It is not possible to draw any close parallel between the two pieces of work except to state that marked results were secured in both instances, in transpiration in the one case and yield in the other. Results secured by Mains (*5*), which are given in table 1, show a distinct loss in yield and kernel weight in the Webster variety when leaf rust inoculation produced only flecking instead of pustulation. The definite losses noted in the present work became evident under conditions of the high 1935 temperatures. There was no way of measuring the damage that might have arisen in 1934.

Chlorosis of the wheat leaf, which often proceeds to further tissue injury, is commonly observed in various wheat varieties but in different degrees. In some cases yellowing apparently results from physiological disturbances, as evidences of infection are not noticeable. Selections from certain crosses seem inclined in this direction. In other cases flecking and the resulting necrosis are obviously due to invasion of rust spores. It is not apparent from the literature that much attention has been paid to leaf chlorosis either as to its cause or its effect upon yield. Probably one reason for this last-named condition is the difficulty of securing quantitative measurements resulting from injury to the green leaf areas. The present study leads to the belief that differences in temperature have a marked influence upon the relationship of such injury to yield and other characters. A rather common opinion, expressed by Richardson (*8*) and others, is that wheat varieties having narrow leaves and scanty foliage give

better returns under dry conditions than do varieties with more ample foliage. This being true, one might presume that with the dry seasonal conditions which prevailed in 1935, and with the families of hybrids discussed in this paper, which have rather well-developed leaf blades, a loss of some of the leaf tissue by necrosis would not influence the character of grain yield. Evidently such was not the case, for a reduction in kernel weight and yield was associated with injury to the leaf.

SUMMARY

By comparing 20 hybrid selections from each of 4 Ceres × (Hope × Florence) families of wheat grown during 1934 and 1935, it was possible to measure the differential effect of leaf rust under two conditions of temperature. The maximum average of leaf rust for any family was only 13 percent.

The family with high resistance to leaf rust and to flecking maintained its yield during 1935, as compared with 1934, in the face of higher temperatures. A second family, resistant to leaf rust but somewhat susceptible to flecking, showed a loss of 16 percent in yield from 1934 to 1935. Two other families, suffering more from leaf rust and from flecking than the two families just mentioned, showed losses of 19 and 28 percent as compared to 1934.

The losses in weight per 1,000 kernels corresponded closely to losses in yield, and this correspondence indicates that the losses in yield were due mainly to reduced size of kernel. Almost no shriveling of the kernel was observed. Weights per bushel were less in 1935, but the decreases here were decidedly smaller than for yield and weight per 1,000 kernels. Heat alone probably affected kernel and bushel weight somewhat adversely.

The two families showing resistance to, or freedom from, leaf rust had a higher protein content in 1935, as compared with 1934, while the two susceptible families showed no increase. The data clearly indicate that the presence of leaf rust reduced the protein content.

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THE INFLUENCE OF COOKING AND CANNING ON THE VITAMIN B AND G CONTENT OF LEAN BEEF AND PORK [1]

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INTRODUCTION

As knowledge of vitamins and their importance in nutrition has broadened, it has become increasingly important to determine, not only the distribution and relative quantities of the several vitamins in raw foodstuffs, but also their vitamin potency as commonly prepared for human or animal consumption. It is well known that such processes as drying, cooking, and canning may alter markedly the vitamin potency of foods, the extent of alteration depending on the processes used and the particular vitamins contained in the food.

Meats have an important place in the American diet. Properly prepared, they furnish protein, energy, certain minerals, notably iron, and vitamins in a palatable and satisfying form. The importance of meat as a source of the vitamin G is well known.

The principal vitamins in lean meat are the antineuritic vitamin B (B_1) and the vitamin G (B_2) complex. Vitamin B is rather easily destroyed by heat, whereas vitamin G is stable, even at fairly high temperatures. Since beef and pork are commonly cooked or canned for human consumption, it seemed worth while to determine what effects, if any, these processes have on their vitamin potency.

PREVIOUS WORK ON VITAMINS B AND G IN MEAT

Comparatively little work had been done on the vitamin content of meats when this project was planned. Most of the work reported had been on raw meats, and involved the vitamin B complex, rather than the separate vitamins B and G.

With pigeons as test animals, Hoagland (6)[3] had found the muscle tissues of the mature ox, calf, and lamb relatively poor to fair in vitamin B, when fed in the form of dried-meat preparations. He found also that hog muscle was richer in vitamin B than ox or sheep muscle, 15 percent in the diet being sufficient to protect pigeons from polyneuritis. Baked pork tenderloin was practically as rich in vitamin as the uncooked muscle, but cooked ham had a somewhat lower value than the raw product. Later Hoagland (7) reported that dried hog muscle added to a diet of polished rice, as 5, 6.6, and 10 percent of the diet, prevented polyneuritis in pigeons, showing pork to be a good source of vitamin B.

If, as indicated by Seidell (*12*), pigeons do not need vitamin G, but are very sensitive to vitamin B deficiency, then the foregoing work was, in reality, a measure of the relative amounts of the antineuritic factor vitamin B only, and not a measure of vitamin G or the vitamin B complex.

In 1926 Goldberger and his associates (*5*) published results of extensive experiments on both man and animals. In their studies on man, they found that a daily allowance of 200 g of fresh, lean beef, freed from visible fat, gristle, and tendons, when finely chopped and mixed with a little water, seasoned with salt and quickly brought to a boil, gave full protection against pellagra.

Their studies on pellagra in man were supplemented by extensive experiments with albino rats. The tests with rats furnished evidence that the so-called "water-soluble B" consisted of at least two factors. One was the antineuritic heat-labile factor and the other, a heat-stable factor, was probably the same as the pellagra-preventing factor (P-P) found in meat and yeast.

Shortly after the present project was planned, but before work was started, Hoagland (*8*) (1929) published a report of tests with both pigeons and rats. He found that 5 percent of dried lean pork protected pigeons against polyneuritis and loss in weight for 8 weeks and longer. Both fresh and smoked hams were found to be equally good sources of the antineuritic vitamin B. Beef was less potent in the antineuritic vitamin B, since it required 35 to 40 percent of the dried beef in the diet to protect pigeons against polyneuritis for 8 weeks.

In his work with rats, Hoagland did not test for vitamins B and G separately, but measured the combined effect of the "water-soluble vitamins." His experiments indicated that pork was a good source of the water-soluble vitamins, but not so good as brewer's yeast. He states (*8, p. 446*):

Lean beef contained much less water-soluble B vitamins than lean pork. From 40 to 70 percent of dried fresh beef was required for excellent growth in rats as compared with 15 to 25 per cent of dried lean pork or 5 per cent of dried brewer's yeast.

Later, Hoagland and Snider (*10*) reported work on the relative quantities of growth-promoting vitamin G in beef, pork, lamb, beef spleen, beef liver, pork liver, and beef kidney. On the basis of air-dried, fat-free material, beef, pork, and lamb appeared to contain approximately the same quantities of vitamin G; 15 to 20 percent of either in the diet being sufficient for excellent growth in rats.

BASAL DIET FOR VITAMIN B DETERMINATIONS

After considering a number of commonly used basal diets for vitamin B determinations, it appeared that the basal diets used by Goldberger and his associates (*5*), and by Hoagland (*8*) were essentially alike and satisfactory for this work.

The following basal diet, free from vitamin B, was adopted and found satisfactory:

Percent

Purified casein _____ 20
Crisco _____ 8
Cornstarch _____ 51
Cod-liver oil _____ 2
McCollum's salt mixture no. 185 _____ 4
Autoclaved baker's yeast _____ 15

The casein, a lactic acid-precipitated product, was extracted with dilute acetic acid, essentially according to the recommendations of Steenbock,[4] but as tests showed that the first lot of casein prepared still contained some vitamin B, the method was modified to insure complete extraction. The procedure found satisfactory and adopted was to extract the casein (15 pounds in a large muslin sack) with dilute acetic acid (about 100 cc of glacial acetic acid to 60 liters of water) in a 25-gallon wooden tub for 12 days. The casein was thoroughly drained and the solution changed once a day. Following this period, the casein was washed with distilled water for 4 days. Each day the casein was drained and fresh water added. The extracted casein was then dried in an electric oven at 130° F. and ground to pass through a 20-mesh sieve.

Crisco was purchased on the market as required. The cornstarch was the ordinary household product and was used without further treatment. One lot of cornstarch was extracted with dilute acetic acid similarly to the casein, but as no difference in results was observed, no further extractions were made. The cod-liver oil was a high-grade product of tested potency.

A pure dehydrated bakers' yeast was used throughout. It was treated and autoclaved according to the method of Chase (*2*) and Chase and Sherman (*3*). It was dried in a cool air-drying apparatus (*11*). According to Chase, 15 percent of the dried autoclaved yeast in the basal diet would furnish an adequate amount of vitamin G without vitamin B. This was confirmed in feeding tests by the authors.

Numerous tests indicated that this diet was satisfactory for the quantitative estimation of vitamin B. The negative control rats, on the basal B-free diet alone, became depleted in 13 to 15 days with an average gain of 20 to 25 g, developing polyneuritis, which was followed by death, in 18 to 28 days after depletion. The positive controls fed the B-free diet plus a known source of vitamin B made normal gains over an indefinite period.

BASAL DIET FOR VITAMIN G DETERMINATIONS

In choosing a basal diet for the vitamin G determinations, it appeared that the alcoholic extract of white corn as used by Goldberger and his associates (*5*) and by Hoagland and Snider (*9*) would be satisfactory as a source of vitamin B, free or practically free from vitamin G.

As different investigators had used alcohols of varying strength, it seemed desirable to conduct a number of preliminary tests. These tests confirmed the findings of others in showing that extracts of white corn made either with 80- or 85-percent alcohol, by weight, were potent in vitamin B and free, or practically free, from vitamin G. Extracts made with 95-percent alcohol contained no appreciable amounts of vitamin B.

These tests indicated also that the extract of corn made with 85-percent alcohol, by weight, and dried on cornstarch so that 1 g of extract was equivalent to 4 g of corn, furnished enough vitamin B for normal growth when included as 7 percent of the diet. Feeding the extract at levels of 10 and 13 percent did not increase the rate of gains.

[4] Steenbock, H. Personal communication to authors.

While these tests were in progress, a commercial vitamin B preparation appeared on the market. In this preparation the vitamin extract was dried on cornstarch so that 5 percent in the diet should furnish adequate amounts of vitamin B. However, the results of feeding tests indicated that 5 percent of the preparation in the diet did not always furnish enough vitamin B, but that 10 percent not only furnished adequate amounts but allowed a margin of safety.

Comparative tests showed that 1 g of the commercial vitamin B preparation was equivalent to 1 g of the 85-percent alcohol extract of corn in which 1 g of extract represented 4 g of corn, and that 7 percent of either in the diet furnished enough vitamin B for normal growth. Since the commercial preparation was found satisfactory, and its use simplified the procedure, it was adopted in preference to the alcoholic extract of corn and was employed in all the tests of the meat preparations.

On the basis of these preliminary tests, the basal vitamin G-free diet adopted was the same as the basal vitamin B-free diet except that 10 percent of the commercial vitamin B preparation and 5 percent of added cornstarch replaced the 15 percent of autoclaved yeast in the vitamin B-free diet.

EXPERIMENTAL PROCEDURE

GENERAL METHOD

The general plan followed in this series of experiments, to determine the vitamin B and G potency of raw, cooked, and autoclaved meats, was the usual rat-growth procedure.

Young albino rats 28 to 30 days old, from known parentage, were placed in large cages having wire-screen bottoms, and fed as a group until growth ceased. The basal diet used for feeding was adequate, so far as known, in all food factors except the specific vitamin to be studied.

The preliminary depletion period was followed by an 8-week experimental period, during which the animals were kept in individual cages with wire bottoms, and fed the basal diet with the meat preparations included in varying percentages or in definite amounts as supplements to the basal diet. The rats had free access to distilled water. Weekly records were kept of live weights, food intake, food waste, and symptoms of vitamin deficiency.

At the end of the 8-week experimental period, all surviving animals were etherized and autopsied, and notations were made of both external and internal conditions.

Two series of feeding tests were made for vitamin B in testing the meat preparations for vitamin potency. In the first series, the dried meats were fed as varying percentages of the basal diet. This was to determine approximately how much of the dried meat was required to maintain an average growth rate of 3 g per week over a period of 8 weeks, which is the growth rate suggested by Chase and Sherman (*3*) and Borquin and Sherman (*1*) for the quantitative determination of vitamins B and G.

In the second series, the different meat preparations were fed in definite amounts separately from the basal diet and not as a percentage of the diet. A modification of the paired system of feeding was used, whereby the rats were selected and matched in sets of three, as

nearly as possible, of the same sex, age, and weight. The rats of each
set of three were distributed, one each, to the groups fed the raw,
cooked, and autoclaved meats. All three rats in the same set were
fed a weighed amount of basal diet, restricted to the level of the rat
eating the least, plus supplements of the raw- cooked, and autoclaved
meat preparations, respectively. Although this adaptation of the
paired feeding method has the disadvantage of keeping the food
intake of some rats below the amount they would otherwise eat, it
has the distinct advantage of limiting the errors in the comparison
of the three meat preparations. Differences in growth of the rats on
raw, cooked, and autoclaved meats can be attributed to differences
in the quantitative vitamin potency of the meats, if all variables in
food intake are controlled.

<div style="text-align:center">THE MEAT PREPARATIONS</div>

The meats used for testing were fresh hams and fresh beef round.
Enough meat was prepared at one time to carry through an entire
unit of the experiment. No attempt was made to determine the origin
of the meat. Two lots of both beef and pork were tested. The lean
meat was separated by hand from the visible fat and connective tissue
and was then ground three times through a meat grinder of the plate
type and thoroughly mixed. The ground meat was divided into
three portions and treated as follows:

(1) Raw meat: Dried and finely ground.

(2) Cooked meat: The ground meat was heated and stirred in a
double boiler until it reached a constant temperature of approximately
90° C., then dried and finely ground.

(3) Autoclaved meat: The raw ground meat was sealed in no. 2 tin
cans without water and processed for 70 minutes at 10 pounds pres-
sure in a pressure cooker or autoclave.[5] The cans were packed as
they would be packed for home canning, and not evacuated. After
processing, the cans were opened and the meat was dried and finely
ground. No determinations were made of the temperature attained
by the meat inside the cans.

All meat samples were air-dried in glass plates on a cool air-drying
apparatus designed and described by Hopper (*11*). The meat juices
which escaped from the meat upon cooking were dried with the meat.

The temperature of the meat samples during drying usually ranged
from 9° to 14° C. In 20 to 22 hours the meat was sufficiently dry for
grinding. It was then finely ground and further dried in vacuo over
sulphuric acid. The dried meats were placed in half-pint glass fruit
jars, the rubbers moistened with glycerin, and the covers clamped in
place. The fruit jars were then placed in vacuum desiccators and
thoroughly evacuated. When the desiccators were opened, the covers
automatically clamped tight, thus sealing the dried meat under par-
tial vacuum. The meat preparations so sealed were kept in an electric
refrigerator until used. At the low temperature and with the speed
at which the drying was accomplished, it is believed that the mini-
mum oxidation took place and that storage under vacuum prevented
deterioration upon standing.

When the jars were opened for use, the raw dry meat was found to
have retained its deep reddish-brown color and the odor of raw meat.

[5] The processing time and pressure were erroneously stated as 60 minutes and 15 pounds, respectively, in the preliminary report 4.

The cooked and autoclaved meats had a brownish-gray color and the odor of a good roast. The flavor of the different preparations was retained in the dried product.

CHEMICAL ANALYSES OF MEATS

Analyses were made of the dried pork and beef preparations to determine the water, protein, and fat content. The average composition of all samples, on a water-free basis, is given in table 1.

TABLE 1.—*Average percentages of protein and fat in water-free meat preparations raw, cooked, and autoclaved*

Meat and condition	Protein	Fat
	Percent	*Percent*
Pork:		
Raw	84.98	10.90
Cooked	85.98	10.34
Autoclaved	86.35	10.38
Beef:		
Raw	92.60	3.85
Cooked	91.75	3.74
Autoclaved	91.64	3.64
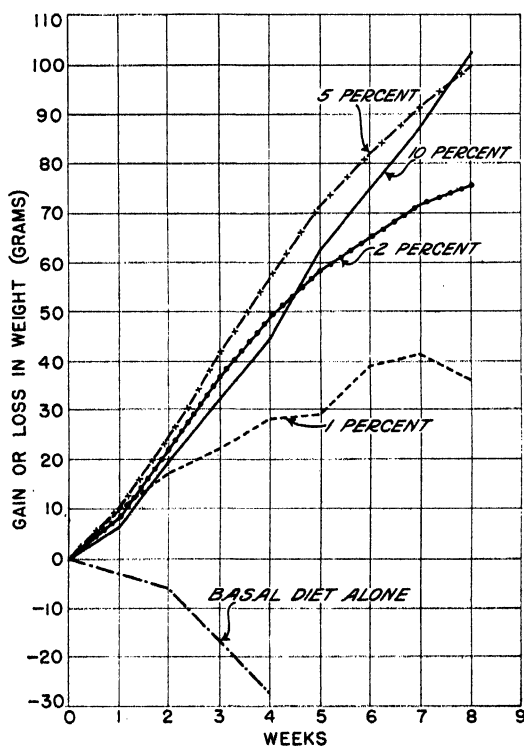
FIGURE 1.—Composite growth curves of rats on the basal diet alone and on the basal diet supplemented with different percentages of dried raw lean pork to provide vitamin B. Two rats were fed the basal diet alone, six received 1 percent, six received 2 percent, nine received 5 percent, and two received 10 percent of pork in their diet. Series 1.

Through a misunderstanding, the water content of the fresh meats in relation to the dried preparations was not determined on all preparations, but determinations on several of the different lots indicated that 1 part of dried product was equal to 3.3 to 4 parts, by weight, of the fresh meat.

VITAMIN B IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN PORK

SERIES 1: DRIED PORK FED AS A PERCENTAGE OF THE DIET

To determine at what level the dried lean pork should be fed to produce the desired gain of 3 g per week during the test period, four groups of rats were fed the dried raw, cooked, and autoclaved pork as 1, 2, 5, and 10 percent of the total food intake. Adjustments were made in the protein and fat to maintain approximately a constant level of 20 percent of protein and 8 percent of fat in the diet. These adjustments for protein and fat were not made in later experiments

where the supplements were fed separately from the basal diet. Wherever the meat preparations were fed as a percentage of the diet, they replaced equal amounts of starch in the basal diet.

The composite growth curves for the groups on the raw dried pork are shown in figure 1.

It is worthy of note that the group on 10 percent of pork made no better gains than the group on 5 percent. The rats on 2 percent of pork gained approximately twice as much as those on 1 percent, but considerably less than those on 5 percent. All the rats fed the different levels of uncooked pork survived the entire 56-day period without any definite polyneuritic symptoms.

Table 2 gives the average weights, gains, and food consumption of all the different groups of rats.

TABLE 2.—*Average weights, gains in weight, and food consumption of groups of rats fed various percentages of dried lean pork, raw, cooked, or autoclaved, as a source of vitamin B in series 1*

| Percentage of dried pork in diet | Rats | Average time on diet | Initial weight | Final weight | Total gain | Average per week | | | | Gain per gram of pork fed | Gain per gram of total food |
						Gain	Basal diet	Pork fed	Total food		
	No.	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1, raw	6	56	85.5	122.0	36.5	4.6	46.1	0.47	46.6	9.79	0.099
1, cooked	[1] 5	56	85.8	118.4	32.6	4.1	46.1	.47	46.5	8.72	.088
1, autoclaved	[1] 5	56	92.0	127.2	35.2	4.4	47.3	.48	47.7	9.17	.092
1, cooked	[2] 6	52.5	87.2	108.3	21.1	2.8	43.1	.44	43.5	6.36	.064
1, autoclaved	[2] 6	52.7	91.3	114.0	22.7	3.0	43.2	.44	43.6	6.82	.069
2, raw	6	56	78.8	154.5	75.6	9.5	51.1	1.04	52.2	9.13	.182
2, cooked	6	56	76.8	150.8	74.0	9.2	51.6	1.05	52.7	8.76	.175
2, autoclaved	6	56	78.5	151.2	72.7	9.1	50.8	1.04	51.9	8.75	.175
5, raw	9	56	87.6	187.7	100.1	12.5	54.8	2.89	57.7	4.33	.217
5, cooked	8	56	80.5	180.6	100.1	12.5	55.9	2.94	58.8	4.25	.213
5, autoclaved	6	56	79.0	170.3	91.3	11.4	54.6	2.88	57.5	3.96	.198
10, raw	2	56	114.5	218.0	103.5	12.9	57.5	6.39	63.8	2.02	.202
10, cooked	2	56	74.0	176.5	102.5	12.8	53.0	5.89	58.9	2.17	.217

[1] Average for the rats that completed the full 56-day period. 1 rat in each group on the 1-percent level of cooked and autoclaved pork died of polyneuritis.
[2] Average for all rats on test.

The data of table 2 show no definite destruction of vitamin B as a result of cooking or autoclaving the pork. However, on 1 percent of dried pork in the diet, one rat in each group fed the cooked and autoclaved pork died of polyneuritis near the middle of the 56-day period. One or two other rats in each of these two groups had rough coats and showed signs of nervousness. This was interpreted as possibly due to a partial destruction of vitamin B.

The average weekly intake of pork on the 1-percent level was approximately 0.45 g, and this amount was chosen for the tests of series 2 on pork.

SERIES 2: DRIED PORK FED SEPARATELY FROM THE BASAL DIET

Eight sets of three matched rats each were fed weighed amounts of the basal diet plus 0.45 g weekly of the dried raw, cooked, or autoclaved pork. To facilitate the weighing of these small amounts of pork, it was mixed with cornstarch, so that 1.5 g of the mixture represented 0.45 g of meat. The weekly portion of supplement was weighed out at the first of the week and fed in three approximately equal feedings. The comparative gains of the rats on the different preparations are shown in figure 2.

It appears from figure 2 that cooking destroyed some of the vitamin B and that autoclaving destroyed more, but not all, of the vitamin.

All of the 24 rats fed in series 2 survived the full experimental period and none showed any definite symptoms of vitamin B deficiency. As a rule, the rats on the autoclaved pork ate the least of the basal diet. Since the food intake in each set of three matched rats was restricted to the rat eating the least, the food intake of the rats on the raw and cooked pork was thereby largely restricted to the level of those fed the autoclaved pork. The principal data for series 2 are given in table 3.
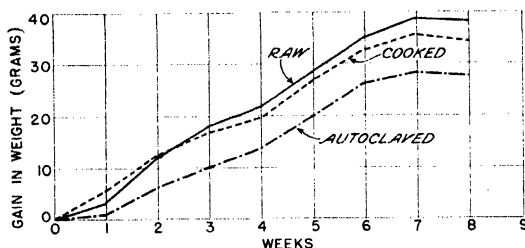
FIGURE 2.—Composite growth curves of three groups of eight rats each receiving 0.45 g of raw, cooked, and autoclaved canned lean pork, respectively, per rat weekly, fed separately from the basal diet in three equal portions to provide vitamin B. Series 2.

Table 3 shows that the food intake was virtually identical on the raw and cooked pork, but averaged about 2 g less per week on the autoclaved pork. The smaller food intake of the rats fed the autoclaved pork was probably due to a partial destruction of the appetite-stimulating vitamin B or some unknown factor. The smaller gains made by the rats on the autoclaved pork are only in part accounted for by the smaller food intake.

Since the total food intake was more accurately controlled in series 2, more weight is given to the results of these tests than to those in which the meat was fed as a percentage of the diet.

TABLE 3.—*Average weights, gains in weight, and food consumption of groups of eight rats fed three equal portions of dried pork, raw, cooked, or autoclaved, separately from their basal diet and at a level of 0.45 g per rat per week, as a source of vitamin B, in series 2 tests lasting 56 days*

| Kind of pork fed | Initial weight | Final weight | Total gain | Average per week | | | | Gain per gram of pork fed | Gain per gram of total food |
				Gain	Basal diet	Pork fed	Total food		
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Raw	77.4	115.0	37.6	4.7	39.3	0.45	39.8	10.44	0.118
Cooked	77.4	110.8	33.4	4.2	39.7	.45	40.2	9.33	.104
Autoclaved	79.3	106.9	27.6	3.5	37.5	.45	38.0	7.78	.092

VITAMIN B IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN BEEF

SERIES 1: DRIED BEEF FED AS A PERCENTAGE OF THE DIET

Thirty-seven rats were fed the vitamin B-free basal diet until depleted and then separated into groups and fed the dried raw lean beef at levels of 2, 5, 7, 10, and 15 percent of the diet to determine the amount of meat required to produce the desired gain of 3 g per week. No cooked or autoclaved beef was fed in this series.

The composite curves of gains or losses by weeks are shown in figure 3, based on the averages of the rats in each group that survived the full 8-week period.

The response of the individual rats to the different levels of beef in the diet varied considerably, especially in some groups, in spite of the fact that the rats were from the same stock and had received the same treatment. These variations are not apparent from the graphs or tables, but are significant in the interpretation of the data and, consequently, are given in some detail.

All the rats fed 2 and 5 percent of dried beef in the diet developed polyneuritis, which resulted in the death of one rat in the 2-percent group after 26 days, and two rats in the 5-percent group. One of these rats died after 8 and the other after 27 days on the test. The remaining rats in each group recovered rapidly when fed yeast.

Three of the nine rats that were started on the 7-percent level of dried beef died after 11, 16, and 19 days, respectively, on the diet. One of the remaining six was fed yeast from the fifth week, but it made only fair recovery. The five rats that survived the full 8-week period made small, but rather uniform, gains during the first 5 weeks, followed by accelerated gains during the last 3 weeks. No explanation for the accelerated gains is apparent.

Three of the twelve rats started on the 10-percent level of dried beef died after 14, 23, and 45 days, respectively, and two rats after reaching a weakened condition, improved markedly when fed yeast. The remaining seven rats, on 10 percent

Figure 3. Composite growth curves of rats fed the basal diet supplemented with different percentages of dried raw lean beef to provide vitamin B. Two rats received 2 percent, three received 5 percent, five received 7 percent, seven received 10 percent, and five received 15 percent of beef in their diet. The rats fed at the 2- and 5-percent levels were near death when yeast was added to their diets as indicated by the asterisk (*) on the graph.

of beef, made virtually identical gains with those on 15 percent, during the first 4 weeks of the test period, but, as a group, they made practically no gains during the last 4 weeks. An inspection of the individual data shows that, for no apparent reason, four of the seven rats gained more or less consistently throughout the test period, reaching an average weight of 126 g as compared to 108 g reached by the five rats on 7 percent of beef and 145 g reached by the five rats on 15 percent of beef. One of the three remaining rats made only a small gain and the other two lost weight during the last half of the test period, thus making the average weight of these three only 74 g at the end of the test.

Six rats were placed on the 15-percent beef diet, but one died at 31 days, leaving five that completed the full period, with fairly steady and uniform gains throughout.

Table 4 gives a summary of the average weights, gains or losses, and food consumption of the rats at the different levels of beef feeding.

TABLE 4.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed various percentages of dried raw beef as a source of vitamin B in series 1*

| Raw beef in diet (percent) | Rats | Average time on diet | Initial weight | Final weight | Total gain or loss | Average per week | | | | Gain or loss per gram of beef | Gain or loss per gram of total food |
						Gain or loss	Basal diet	Beef fed	Total food		
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
2	[1] 3	29.7	73.0	55.0	−18.0	−4.3	29.0	0.6	29.6	−7.17	−0.145
5	[2] 7	23.9	63.4	58.4	−5.0	−1.9	32.2	1.7	33.9	−1.12	−.056
7	[3] 5	56.0	70.0	107.6	37.6	4.7	42.3	3.2	45.5	1.47	.103
10	[4] 7	56.0	65.9	102.4	36.5	4.6	39.6	4.4	44.0	1.05	.105
15	[5] 5	56.0	65.5	145.0	79.4	9.9	44.0	7.8	51.8	1.27	.191

[1] Average of all rats in group up to time of feeding yeast; 1 of the 3 rats died before yeast was fed.
[2] Average of all rats in group up to time of feeding yeast; 2 of the 7 rats died before yeast was fed.
[3] 9 rats started; 3 died and 1 was fed yeast; averages are for remaining 5 rats.
[4] 12 rats started; 3 died and 2 were fed yeast; averages are for remaining 7 rats.
[5] 6 rats were started but 1 died; averages are for remaining 5 rats.

From the results obtained, it was difficult to determine how much beef would be required to furnish enough vitamin B to maintain an average gain of 3 g per week, but, apparently, 7 percent in the diet, or 3.2 g of dried beef per week, was about the lower limit.

SERIES 2: DRIED BEEF FED SEPARATELY FROM THE BASAL DIET

Sets of three matched rats each were fed on equal weighed portions of dried raw, cooked, and autoclaved beef, fed separately from the basal diet to insure more uniform consumption of the beef. The basal food intake was also kept as uniform as possible, but it was found that the rats on the autoclaved beef almost invariably decreased their food intake as the period progressed. Whenever a rat on the autoclaved beef ate less than 3 g of food per day, it was dropped from the set and the food intake equalized between the rats on the raw and cooked beef. All the rats relished the

FIGURE 4.—Composite growth curves of two groups of eight and one group of nine rats receiving 4.5 g of raw, cooked, and autoclaved canned lean beef, respectively, per rat weekly, fed separately from the basal diet in three equal portions to provide vitamin B. Series 2.

raw and cooked beef, but those on the autoclaved beef showed a consistent dislike for it. Some of the autoclaved beef was frequently left uneaten or wasted from the food cup. It was, therefore, impossible to keep the food intake alike for all the rats.

No such difficulty existed with the pork, possibly due to the fact that the pork was fed in small amounts (less than 0.5 g per week) mixed with cornstarch, or to the larger amounts of vitamin B contained in the autoclaved pork as compared to the beef, or to a loss of the appetite-stimulating factor, vitamin B. The last-mentioned seems the most likely explanation, for when adequate amounts of
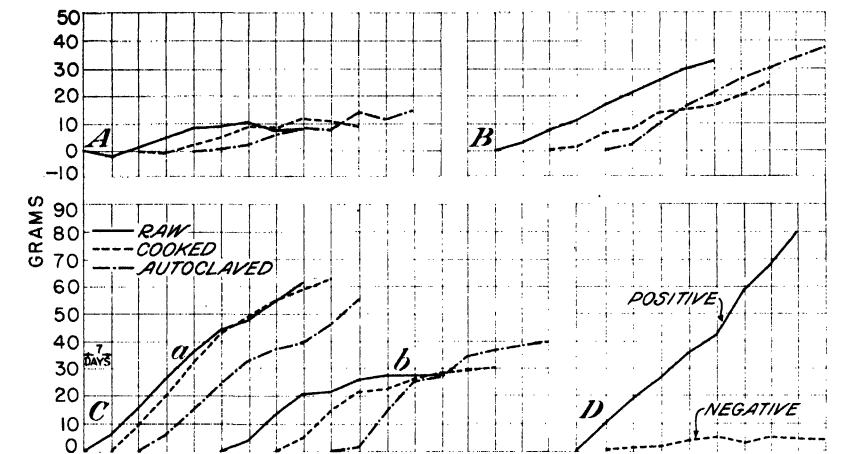
FIGURE 5.—Composite growth curves of rats receiving various quantities of dried pork added separately to their diets to provide vitamin G: *A*, three groups of eight rats each, fed 1 g of pork per rat per week; *B*, three groups of five rats each, fed 1.5 g of pork per rat per week; *C*, three groups of six rats each in series 1 (*a*) and three rats each in series 2 (*b*), fed 2 g pork per rat per week; *D*, groups of four rats on positive and six on negative control diet.

vitamin B were furnished, as in testing for vitamin G, the rats ate the autoclaved beef readily.

Because of the results of series 1, levels of 4, 4.5, and 6 g of dried beef weekly were chosen for series 2 in testing the relative potency of the three preparations for vitamin B.

The composite weight curves of the rats fed 4.5 g of beef per week are shown in figure 4.

TABLE 5.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed three equal portions of dried beef, raw, cooked, or autoclaved, separately from their basal diet and at levels of 4, 4.5, and 6 g per rat per week, as a source of vitamin B in series 2*

Amounts and kind of beef fed weekly	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain or loss per gram of beef fed	Gain or loss per gram of total food
						Gain or loss	Basal diet	Beef fed	Total food		
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
4 g:											
Raw	5	56	89.0	112.0	23.0	2.9	32.5	4.0	36.5	0.73	0.079
Cooked	5	56	88.2	118.4	30.2	3.8	33.4	4.0	37.4	.95	.102
Autoclaved	5	39	87.6	60.6	−27.0	−5.7	21.2	2.7	23.9	−2.11	−.238
4.5 g:											
Raw	8	54.3	80.9	111.0	30.1	3.9	33.3	4.5	37.8	.87	.103
Cooked	8	54.3	81.4	104.4	23.0	3.0	31.9	4.5	36.4	.67	.082
Autoclaved	9	50.0	84.3	67.3	−17.0	−3.8	23.1	3.9	27.0	−.97	−.141
6 g:											
Raw	2	56.0	72.5	118.5	46.0	5.8	35.2	6.0	41.2	.97	.141
Cooked	2	56.0	69.5	112.5	43.0	5.4	33.7	6.0	39.7	.90	.136
Autoclaved	2	50.5	71.0	57.0	−14.0	−2.1	20.9	4.2	25.1	−.50	−.084

Figure 4 shows that the rats on the cooked beef did not gain as well as those on the raw beef, and also that the ones on the autoclaved beef lost weight from the start. The average results for all the groups at the different levels of beef feeding are shown in table 5.

The small average weekly gains made by the rats on 4 g of dried raw beef per week are mainly due to the fact that one rat wasted food and lost weight; the other four averaged 3.7 g gain per week, which is practically the same as the gains made by the rats on the cooked beef. Of the five rats on the autoclaved beef, one made a small gain of 3 g in 8 weeks; the other four lost from 32 to 38 g.

Of the eight rats fed 4.5 g of raw beef weekly, one lost 2 g, but the others gained from 28 to 40 g each. Similarly, one of the rats on the cooked beef lost 5 g, while the others gained from 9 to 37 g. If the ones that lost weight are omitted, the average weekly gains per rat are 4.5 g on the raw, and 3.5 g on the cooked beef.

All rats fed 6 g of raw and cooked beef weekly survived the entire 8-week period and made larger average weekly gains than the groups fed at the lower levels. Both of the rats fed the autoclaved beef lost weight. One of the rats died at the end of 45 days on the diet.

With one or two exceptions on the 4- and 4.5-g levels, all the rats on the autoclaved beef lost weight, but evidently the autoclaved beef still contained some vitamin B, since the losses in weight decreased and the survival period increased in the successive groups with the increase in level of beef feeding.

UNITS OF VITAMIN B IN DRIED LEAN PÓRK AND BEEF

The units of vitamin B in the dried lean pork and beef, computed according to the method of Chase and Sherman (*3*), are presented in table 6.

UNITS OF VITAMIN B PER GRAM OF DRIED LEAN PORK

The units of vitamin B in pork, as given in table 6, were computed only at the 1- and 2-percent levels and 0.45 g weekly, as the gains on the 5- and 10-percent levels were too large to furnish a reliable measure.

In series 1, where the pork was fed as a percentage of the diet, there is no clear-cut or definite indication of a destruction of vitamin B as a result of either cooking or autoclaving. However, in series 2, where the pork was fed separately from the basal diet, and where the food was equalized more closely in the different sets of three, there appears to be a rather definite indication that some of the vitamin was destroyed as a result of cooking and autoclaving. From the values found in series 2 alone, it appears that approximately 10 percent of vitamin B was destroyed as a result of cooking and 25 percent as a result of autoclaving the pork. Slightly different results are obtained when the rats on the 1-percent level of pork are included. The weighted average for the rats fed at the 1-percent level of dried pork and those fed 0.45 g per week, separately from the basal diet, is 24 units per gram of dried raw pork. This is equivalent to approximately 7 units per gram of fresh pork. For the cooked pork, the weighted average is 21 units and for the autoclaved pork, it is 19 units per gram of dried product.

TABLE 6.—*Units of vitamin B in variously treated dried pork and beef, as computed from the data by the method of Chase and Sherman*

PORK

· Series, treatment of meat used, and quantity fed	Rats	Average time on diet	Average weekly gains or losses in weight	Vitamin B per gram of meat
Series 1:	*Number*	*Days*	*Grams*	*Units*
1 percent, raw	[1] 6	56.0	4.6	22.8
1 percent, cooked	[2] 5	56.0	4.1	20.5
1 percent, autoclaved	[2] 5	56.0	4.4	21.2
1 percent, cooked	[1] 6	52.5	2.8	14.8
1 percent, autoclaved	[1] 6	52.7	3.0	15.9
2 percent, raw	6	56.0	9.5	21.2
2 percent, cooked	6	56.0	9.2	20.2
2 percent, autoclaved	6	56.0	9.1	20.3
Series 2:				
0.45 g, raw, weekly	8	56.0	4.7	24.5
0.45 g, cooked, weekly	8	56.0	4.2	21.9
0.45 g, autoclaved, weekly	8	56.0	3.5	18.2

BEEF

Series 1:				
7 percent, raw	[3] 5	56.0	4.7	3.4
10 percent, raw	[4] 7	56.0	4.6	2.4
15 percent, raw	[2] 5	56.0	9.9	3.0
Series 2:				
4.0 g, raw, weekly	5	56.0	2.9	1.7
4.0 g, cooked, weekly	5	56.0	3.8	2.2
4.0 g, autoclaved, weekly	5	39.0	−5.7	
4.0 g, raw, weekly	[3] 4	56.0	3.7	2.2
4.5 g, raw, weekly	8	54.3	3.9	2.0
4.5 g, cooked, weekly	8	54.3	3.0	1.6
4.5 g, autoclaved, weekly	9	50.0	−3.8	
4.5 g, raw, weekly	[5] 7	56.0	4.5	2.3
4.5 g, cooked, weekly	[5] 7	56.0	3.5	1.8
6.0 g, raw, weekly	2	56.0	5.8	2.3
6.0 g, cooked, weekly	2	56.0	5.4	2.1
6.0 g, autoclaved, weekly	2	50.5	−2.1	

[1] Averages for all rats in the group.
[2] Average, omitting 1 rat that died in each group.
[3] Average of 5 rats out of 9 that lived through entire period without modification of diet.
[4] Average of 7 rats out of 12 that lived through entire period without modification of diet.
[5] Average, omitting 1 rat in each group that lost weight.

UNITS OF VITAMIN B PER GRAM OF DRIED LEAN BEEF

The units per gram of dried beef in series 1 are slightly higher than the units as determined in series 2. In series 2, at the level of 4 g of dried beef per week, there is no definite indication of a destruction of vitamin B, but at the 4.5-g level there appears to be a destruction of vitamin B as a result of cooking. At the 6-g level, there is also no definite indication of a destruction of vitamin B as a result of cooking, but at all three levels, there is definite indication that autoclaving destroyed at least a part of the vitamin contained in the beef. The dried lean raw beef contained an average of about 2.5 units per gram, which is equivalent to 0.6 unit per gram of fresh beef. From this it is evident that the lean beef contained only about one-tenth as much vitamin B as the pork.

It is not possible from the data to explain why autoclaving destroyed the vitamin B more completely in the lean beef than in the lean pork. The data of table 1 show that the lean pork contained about three times as much fat as the lean beef, and it is suggested that the fat may possibly have aided in protecting the vitamin from destruction.

VITAMIN G IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN PORK

Pork was fed at levels of 1, 1.5, and 2 g per week, as preliminary tests had shown, these levels to be suitable for testing for vitamin G. The pork for the entire week was weighed out in advance and fed as a supplement separately from the basal diet in approximately three equal portions during the week.

Composite growth curves for the different groups are shown in figure 5, and a summary of the data by groups, including the controls, is given in table 7.

TABLE 7.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed dried pork, raw, cooked, or autoclaved, separately from their basal diets and at levels of 1, 1.5, and 2 g per rat per week, as a source of vitamin G*

Amounts and treatment of pork fed weekly for period and series indicated	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain per gram of pork fed	Gain or loss per gram of total food
						Gain or loss	Basal diet	Pork fed	Total food		
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1 g, September to November 1932:											
Raw	8	56	67.9	75.9	8.0	1.0	33.7	1.0	34.7	1.00	0.029
Cooked	8	54	60.1	75.7	9.6	1.2	33.6	1.0	34.6	1.20	.034
Autoclaved	8	56	67.5	81.5	14.0	1.7	33.1	1.0	34.1	1.70	.050
Negative controls	3	56	50.3	73.3	23.0	2.9	33.3	.0	33.3	------	.087
Positive controls	2	56	53.5	117.0	63.5	8.0	43.6	1.0	44.6	8.00	.179
1.5 g, April to July 1933:											
Raw	5	56	38.8	70.8	32.0	4.0	30.6	1.5	32.1	2.67	.125
Cooked	5	56	38.6	62.2	23.6	3.0	27.7	1.5	29.2	2.00	.103
Autoclaved	5	56	38.2	75.0	36.8	4.6	30.9	1.5	32.4	3.07	.142
Negative control	1	56	57.0	69.0	12.0	1.5	29.8	.0	29.8	------	.050
2 g, series 1, June to August 1932:											
Raw	6	56	60.1	121.5	61.3	7.7	37.8	2.0	39.8	3.85	.193
Cooked	6	56	59.3	121.8	62.5	7.8	38.6	2.0	40.6	3.90	.192
Autoclaved	6	56	60.5	116.0	55.5	6.9	36.4	2.0	38.4	3.45	.180
Negative controls	2	56	56.0	55.5	-.5	-.1	26.6	.0	26.6	------	-.004
Positive controls	2	56	45.0	139.5	94.5	11.8	38.2	3.5	41.7	3.37	.283
2 g, series 2, July to September 1933:											
Raw	3	56	63.3	90.6	27.3	3.4	29.2	2.0	31.2	1.70	.109
Cooked	3	56	63.0	93.2	30.3	3.8	31.1	2.0	33.1	1.90	.115
Autoclaved	3	56	63.0	103.0	40.0	5.0	31.2	2.0	33.2	2.50	.151
Negative control	1	56	70.0	75.0	5.0	.6	32.7	.0	32.7	------	.018

It was not possible to conduct all the feeding trials at the different levels simultaneously. Series 1 at the level of 2 g of dried pork weekly was carried out during the first summer, followed by the group on 1 g of pork weekly during the fall of the same year. The following spring the tests at the 1.5-g level were made, followed by series 2 on the 2-g level during the summer.

The data of table 7 show that 1 g of the dried pork preparations failed to support a unit gain of 3 g per week, and that 1.5 g gave slightly more than a unit rate of gain per week. The gains on 2 g of pork in series 1 were nearly twice as great as the gains on 1.5 g, but in series 2 at the same level of pork as in series 1, the gains were similar to those on the 1.5 g of pork. At first it might appear that a loss in vitamin potency had occurred between the tests of series 1 the first summer, and series 2 the following summer; but this seems unlikely

since the tests at the 1.5-g level were made just prior to the tests of series 2. Moreover, no decrease in potency due to storage was observed in the beef under similar circumstances.

The larger food intake by the rats in series 1 as compared to series 2 may explain in part the differences in the gains of the rats in the two series, but it does not explain why the rats on 1.5 g of pork made virtually the same gains as those on 2 g in series 2 where the food intake was practically the same. Possibly differences in the state of depletion of the rats at the start was a factor.

In spite of some inconsistencies in the gains on the raw, cooked, and autoclaved pork in the different groups, and on the different levels of intake, the data fail to show any destruction of vitamin G in the pork when cooked or autoclaved by the methods here used.

VITAMIN G IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN BEEF

The work of Hoagland and Snider (*10*) had indicated that pork and beef have about the same vitamin G potency. With this in
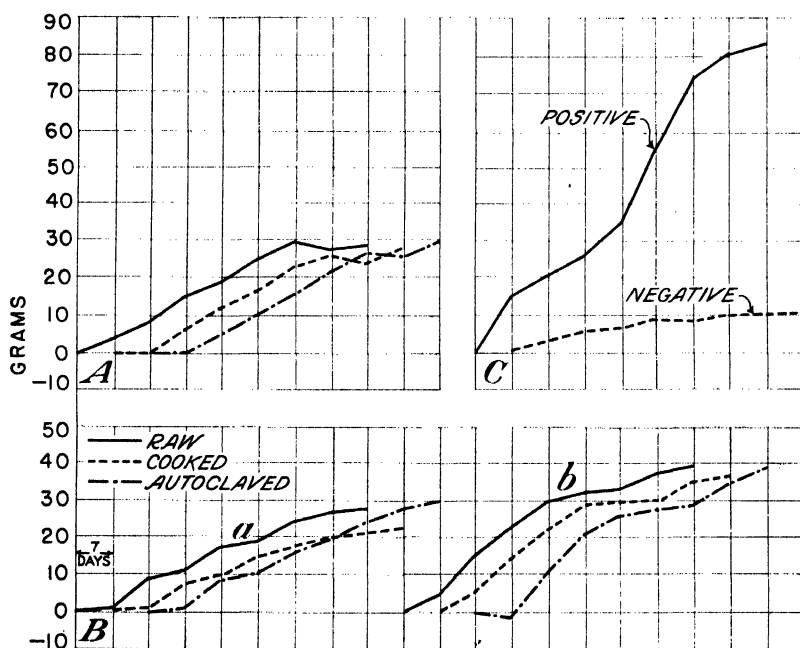
FIGURE 6.—Composite growth curves of rats receiving dried lean beef added separately to their diets to provide vitamin G: *A*, Three groups of 10 rats each fed 1.5 g of beef per rat per week; *B*, three groups of 10 rats each in series 1 (*a*) and 3 rats each in series 2 (*b*) fed 2 g of beef per rat per week; *C*, 1 rat on positive and group of 6 rats on negative control diet

mind the writers fed dried beef only at levels of 1.5 and 2 g per week, as 1 g of pork had been found inadequate.

The composite growth curves of the rats fed in these tests are shown in figure 6, and a summary of the gains and food intake is given in table 8.

TABLE 8.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed dried beef, raw, cooked, or autoclaved, separately from their basal diets and at levels of 1.5 and 2 g per rat per week, as a source of vitamin G*

Amounts and treatment of beef fed weekly for period and series indicated	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain per gram of beef fed	Gain or loss per gram of total food
						Gain or loss	Basal diet	Beef fed	Total food		
1.5 g, March to May 1933:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Raw	10	56	52.0	80.6	28.6	3.6	33.9	1.5	35.4	2.40	0.102
Cooked	10	56	52.4	79.5	27.1	3.4	34.0	1.5	35.5	2.27	.096
Autoclaved	10	56	52.3	81.5	29.2	3.7	33.7	1.5	35.2	2.47	.105
Negative controls	4	56	52.5	65.0	12.5	1.6	31.0	.0	31.0	____	.052
2 g, series 1, December to February 1932–33:											
Raw	10	56	63.0	90.1	27.1	3.4	34.4	2.0	36.4	1.70	.093
Cooked	10	56	61.7	84.2	22.5	2.8	33.9	2.0	35.9	1.40	.078
Autoclaved	10	56	61.4	90.2	28.8	3.6	33.8	2.0	35.8	1.80	.101
Negative controls	1	56	34.0	45.0	11.0	1.4	26.5	.0	26.5	____	.053
Positive controls	1	56	47.0	130.0	83.0	10.4	51.1	2.0	53.1	5.20	.196
2g, series 2, July to September 1933:											
Raw	3	56	59.7	98.7	39.0	4.9	32.7	2.0	34.7	2.45	.141
Cooked	3	56	61.0	97.3	36.3	4.5	32.7	2.0	34.7	2.25	.130
Autoclaved	3	56	62.0	100.3	38.3	4.8	32.2	2.0	34.2	2.40	.140
Negative control	1	56	44.0	42.0	−2.0	−.3	21.3	.0	21.3	____	−.014

The 10 sets of 3 matched rats each fed 1.5 g of dried beef per week separately from the basal diet (table 8) were fed simultaneously with the 5 sets of 3 rats on 1.5 g of dried pork (table 7). The data show that the rats on the raw, cooked, and autoclaved beef gained 3.6, 3.4, and 3.7 g, respectively, as compared to gains of 4.0, 3.0, and 4.6 g by the rats on the pork. These results indicate that the vitamin G potency of the beef and pork preparations was practically the same.

Although there were considerable variations in the rates of gain among the groups, the results indicate no loss of vitamin G potency as a result of either cooking or autoclaving.

UNITS OF VITAMIN G IN DRIED LEAN PORK AND BEEF

The units of vitamin G in the several feeding periods and at the different levels of feeding have been computed according to the method of Borquin and Sherman (*1*), and are given in table 9.

The units of vitamin G per gram of dried pork and beef in table 9 vary considerably between different levels of feeding and between series on the same level, but a comparison of the units per gram of meat fails to show any definite destruction of vitamin G as a result of cooking or autoclaving. Using the data from all the rats fed at the 1.5 and 2 g levels, the weighted average units of vitamin G per gram of dried meat are: 6.9 for the raw, 6.5 for the cooked, and 7.3 for the autoclaved pork, as compared to 4.9 for the raw, 4.4 for the cooked, and 5.1 for the autoclaved beef.

TABLE 9.—*Units of vitamin G in variously treated dried pork and beef as computed from the data by the method of Borquin and Sherman*

PORK

Series, treatment of meat used, and quantity fed weekly	Rats	Average time on diet	Average weekly gains in weight	Vitamin G per gram of meat
	Number	*Days*	*Grams*	*Units*
1 g, raw	8	56	1.0	2.3
1 g, cooked	8	54	1.2	2.8
1 g, autoclaved	8	56	1.7	4.0
1.5 g, raw	5	56	4.0	6.2
1.5 g, cooked	5	56	3.0	4.7
1.5 g, autoclaved	5	56	4.6	7.2
Series 1:				
2 g, raw	6	56	7.7	9.0
2 g, cooked	6	56	7.8	9.1
2 g, autoclaved	6	56	6.9	8.1
Series 2:				
2 g, raw	3	56	3.4	4.0
2 g, cooked	3	56	3.8	4.4
2 g, autoclaved	3	56	5.0	5.8

BEEF

1.5 g, raw	10	56	3.6	5.6
1.5 g, cooked	10	56	3.4	5.3
1.5 g, autoclaved	10	56	3.7	5.8
Series 1:				
2 g, raw	10	56	3.4	4.0
2 g, cooked	10	56	2.8	3.3
2 g, autoclaved	10	56	3.6	4.2
Series 2:				
2 g, raw	3	56	4.9	5.7
2 g, cooked	3	56	4.5	5.2
2 g, autoclaved	3	56	4.8	5.6

SUMMARY OF RESULTS

The main facts brought out in these studies on pork and beef as are follows:

(1) The dried raw lean pork used in the tests was a good source of vitamin B, containing about 24 units per gram of dried material. This is equivalent to about 7 units per gram of fresh pork. The dried raw lean beef contained 2.5 units of vitamin B per gram, equivalent to 0.6 unit per gram of fresh beef. On this basis the fresh lean beef contained less than one-tenth as much vitamin B as the pork.

(2) The lean pork cooked at a temperature not exceeding 90° C. contained 21 units of vitamin B per gram of dried material, representing a loss of 12 percent in potency. Lean beef cooked in the same way contained 2 units per gram of dried material, representing a loss in potency of 20 percent.

(3) Heating the pork in a steam autoclave or pressure cooker for 70 minutes at 10 pounds' pressure destroyed more of the vitamin B than did cooking. The autoclaved pork had a value of about 19 units per gram of dried material, representing a loss in potency of approximately 21 percent. Under similar treatment the vitamin B in the beef was almost completely destroyed.

(4) The vitamin G content of lean pork and beef is nearly the same. The average units per gram of dried material for pork are: Raw 6.9, cooked 6.5, and autoclaved 7.3. For dried beef the units are: Raw 4.9, cooked 4.4, and autoclaved 5.1. The data indicate no destruction of vitamin G as a result of cooking or autoclaving.

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AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT [1]

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INTRODUCTION

There are described in this paper the principles of an automatically operated sand-culture equipment. This equipment is designed to provide (1) the advantages of large-vessel, or flowing-type, water cultures with regard to the maintenance of solution concentration, and (2) the numerous advantages of sand cultures, such as aeration, iron supply, seedling germination, root environment, and elimination of plant supports.

Students of plant nutrition have relied for a good many years on the use of artificially prepared solutions for the development of information on the essential character and toxicity of the salt constituents of the soil solution. In investigations of purely qualitative character, specialized equipment has been largely uncalled for, but in many directions inquiries of this type have now progressed into quantitative fields; accordingly, it has become increasingly important that the methods employed be such that the concentrations of ions presented to plant roots can be maintained within closely fixed limits.

Briefly stated, with the equipment here described the solution is applied by automatically controlled pumps to the surface of free-draining sand cultures at hourly or other selected intervals. The displaced solution returns by gravity to the supply reservoir. Since the capacity of this reservoir is large as compared with the volume of the solution held by the sand, the maintenance of solution concentrations within reasonable limits resolves itself into a matter of periodic replenishment of the water and salt constituents that are removed by the plants. The frequent automatic replacement of the solution held by the sand prevents any noteworthy changes in the concentrations of elements presented to the plant roots. New solutions are recurrently substituted for those in use. Iron is supplied in the form of water-insoluble minerals mixed with the sand. The present paper gives a detailed description of the construction and operation of this widely adaptable sand-culture principle.

DESCRIPTION

GENERAL

A unit of sand-culture equipment comprises a sand culture, a solution reservoir, and a motor pump. Whether small, as for single plants or seedlings in the greenhouse, or large, as for trees carried into the fruiting stage, the sand cultures are always free-draining. The capacity of the solution reservoir should be large as compared with the daily transpiration demands of the plants. The reservoir is set at a level below that of the sand culture. The solution, intermittently

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pumped from this reservoir, is distributed over the top of the sand by suitably arranged perforated pipes, the rate of application being such that a sheet of free water is quickly developed. The volume per application, and therefore the duration of the run, is such that the solution residual from the previous application is displaced either wholly or partly, depending on the volume of the sand and the size of the plants. Drainage returns to the solution reservoir. The equipment is fully automatic. A time clock, with simple adjustments for frequency and duration of flooding, controls a magnetic switch that starts the pumps.

SAND BEDS AND RESERVOIRS

The method for maintaining solution concentrations is applicable to sand cultures of varied characteristics. The type or size of culture vessel depends on the particular experimental purpose served.
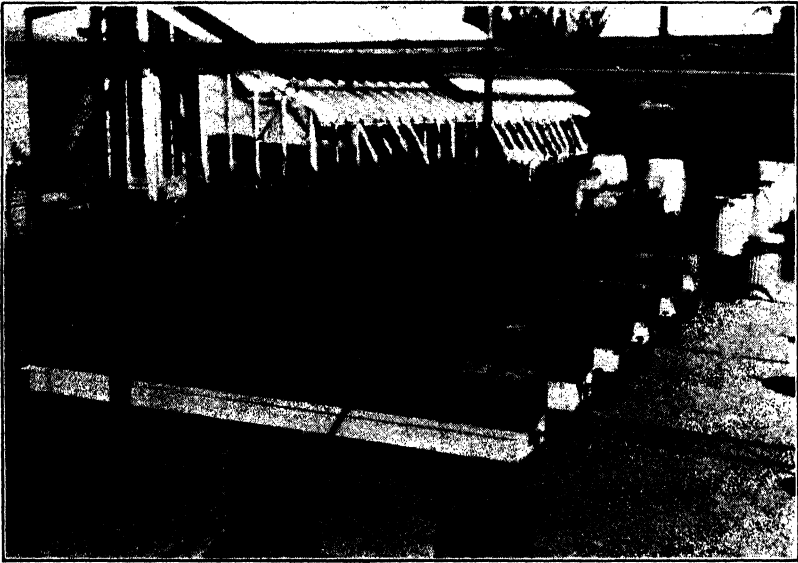
FIGURE 1.—Sand beds of galvanized iron. In each bed there are duplicate plantings of each of four crops in an experiment designed to determine the effect of high and low nitrate concentrations on the tolerance of each of these four kinds of plants to chloride and sulphate salts.

The sand beds described in an earlier publication,[2] and here illustrated as figure 1, have now been equipped with motors, and larger solution reservoirs have been installed. These sand beds, six in number, have surface dimensions of 18 inches by 12 feet. They are 11 inches deep at the sides and 13 inches deep along the center line. The bottom 2 inches of the beds (from the center line) is filled with pea gravel, and above this is placed a ¾-inch layer of coarse sand. The beds are then filled to within 1½ inches of the top with quartz sand. A ¼-inch-mesh screen separates the quartz sand from the coarse drainage sand so that the latter will not be disturbed when the roots of plants are pulled out in harvesting. Beneath the tanks, along the center line, there is riveted and soldered a 2-inch drain roll.

[2] EATON, F. M. A LARGE SAND CULTURE APPARATUS. Soil Sci. 31: 235–241, illus. 1930.

Multiple perforations in the bottom of the sand bed permit drainage into this roll, which in turn drains through a 1-inch pipe to the supply reservoir. A 2-inch overflow pipe connected to the drain roll opens about 1 inch above the surface of the sand. Evaporation and the
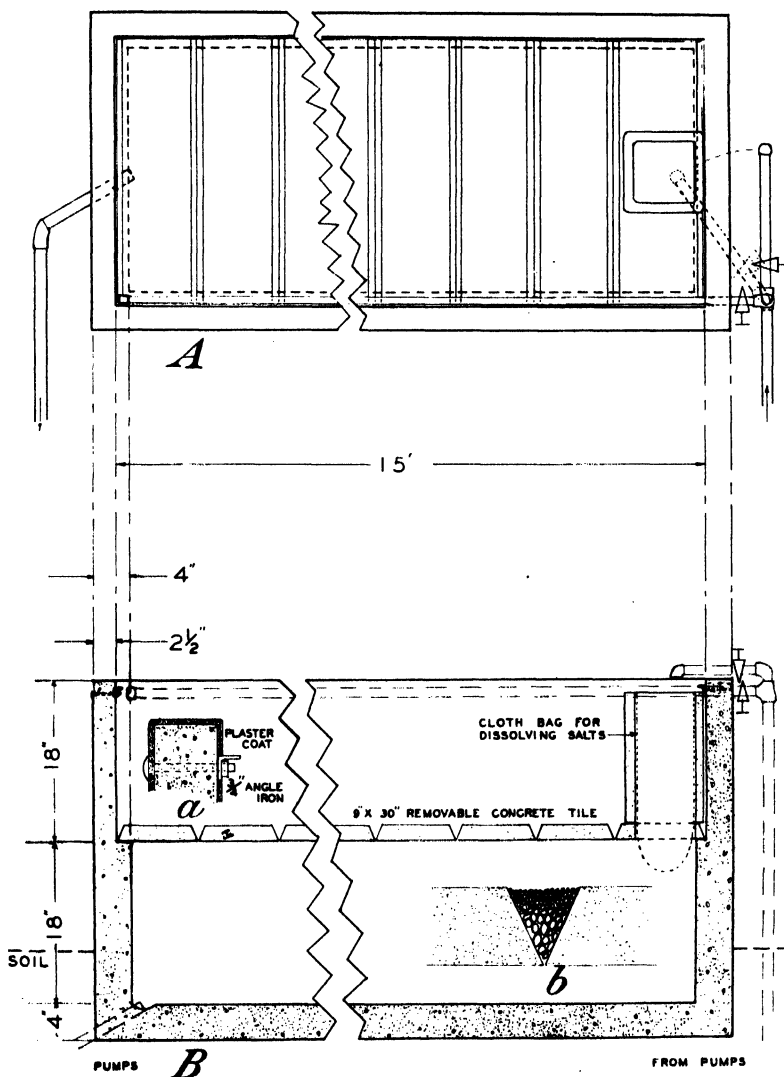
FIGURE 2.—Sections of shallow concrete sand beds to be used for annual crops: *A*, Horizontal section; *B*, vertical section. *a*, Reinforcing angle iron (welded at four corners), which supports perforated metal plates above sand, to suppress evaporation and the growth of algae; *b*, a section at edges of two cross tiles, showing the sand-and-gravel drainage way.

growth of algae are restricted in these beds by perforated galvanized plates supported a little above the surface of the sand. These plates, 12 by 18 inches, are used in pairs that overlap between the rows of plants, the edges next to the plants being turned up about one-half inch to prevent injury to the plant stems.

The sand cultures just described are to be replaced with new equipment of larger dimensions having certain advantages in design. The new cultures (fig. 2), are to be constructed of concrete, with each sand bed and its reservoir poured as a combined unit. The inside surface of the sand beds will be 30 inches wide and 15 feet long, and the depth of the sand will be 13.5 inches. The sand is to be supported above the solution by concrete tile resting on ledges. Each of the reservoirs is to have a capacity of 1,469 liters. The sand, extending to within 2½ inches of the top of the bed, will have a volume of 42.5 cubic feet, or 1, 204 cubic decimeters. Drainage from the sand to the reservoirs is effected through coarse sand and gravel placed between the beveled edges of the supporting tile. An earthen tile 7 inches square that extends from above the sand to an opening made in one of the end supporting tiles provides access to the reservoirs for measuring and renewing the culture solutions. Eight of these culture beds are to be constructed. The solution pumps, operated by belts from a shaft driven by a single motor, will be
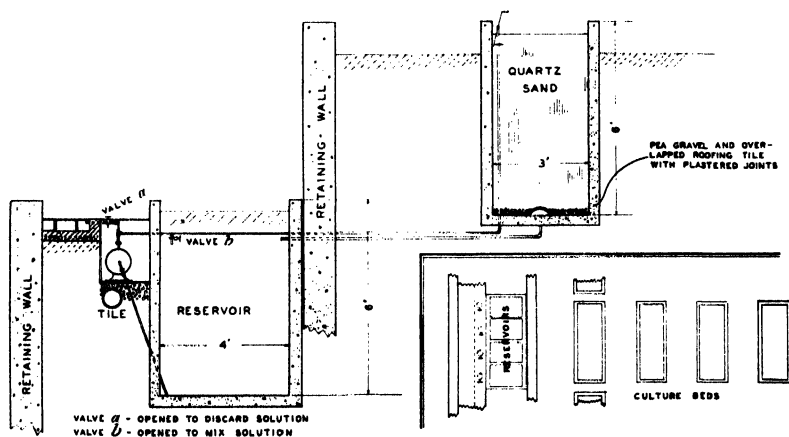
FIGURE 3.—Cross section of one of the deep sand beds with its supply reservoir. The insert shows the arrangement of four of these beds.

assembled in a suitable housing at one end of the experimental enclosure. · The pumps, connected by 1¼-inch pipe with the reservoirs and sand-bed outlets, will have discharges of about 50 liters per minute. It is estimated that this complete equipment, exclusive of the culture sand, will cost about $1,000.

The culture vessels used at the Rubidoux Laboratory, Riverside, Calif., for trees (fig. 3) are constructed of concrete, the walls being 4 inches thick, the inside surface 3 by 10 feet, and the depth 6 feet. These beds, 20 in number, rise 1 foot above the ground and extend 5 feet into the ground. The supply reservoirs rise slightly above the surface of the ground on a lower terrace. A 1-inch layer of pea gravel, placed over the surface of permanent cultures of this type to check evaporation and algae growth, takes the place of the galvanized plates used on the metal sand beds where annual plants are grown.

Four sets of six 80-liter galvanized-metal containers are also in use. Each set of these cans has a common reservoir from which solution is withdrawn and to which the solution returns by free drainage. This

equipment permits of four treatments with six different test crops under each.

It is to be recommended that the supply reservoirs for culture solutions be made large. Both water-retaining capacity of the sand and the expected daily volume loss by transpiration when plants are at full growth should be taken into account in planning for reservoir capacity.

Each of the shallow metal sand beds contains 382 cubic decimeters of sand and retains approximately 160 liters of solution against gravity, and each of the supply reservoirs holds 400 liters of solution, making a total of 560 liters of solution in each system. When plants are at full growth in the control bed, a day's transpiration loss in excess of 60 or 70 liters is regarded as high. If the absorption of salt constituents is neglected, then the concentration of the solution in the system would be increased in the order of 11 percent by 60 liters of transpiration. These supply reservoirs, accordingly, are regarded as being too small.

Per unit of sand surface, the reservoir capacity of the new shallow beds of concrete will be approximately double that of the shallow metal beds.

The capacity of each of the reservoirs of the large cultures for trees (fig. 3) is 1,600 liters. About 500 liters of solution is retained by the sand in each bed. Since the daily transpiration of 4-year-old trees grown in these beds will rarely exceed 100 liters, the daily concentration of the solution in the system will be increased by about 5 percent except possibly on occasional days when the saturation deficit is unusually high.

Rust-resistant galvanized metal will give many years of service if used above ground, particularly when a first coat of red-lead paint is applied inside. If properly protected outside by dressings of lead and asphalt, good underground service can be expected also; but such construction cannot be looked upon as permanent, and slow leakage is difficult to detect. When economy has been necessary, 50-gallon iron oil drums have been used; but these will last only a few years.

In connection with this description of metal beds, the comment may be made that there has been no evidence that plant injury has resulted from the use of red-lead paint applied as a first coat to protect the galvanized-metal surfaces from corrosion. Corn plants have been grown without injury in water cultures when a large mat of excelsior dipped in red-lead paint and dried, was inserted into the mason jars used as culture vessels. Plants were likewise uninjured when as much as 20 parts per million of lead as lead acetate was added to culture solutions. In the plumbing for sand-culture solutions with hydrogen-ion concentrations between pH 6 and 8, bronze and brass valves have been used freely without evidence of plant injury.

As a result of surface tension, the lower several inches of sand in cultures remains almost saturated with water after drainage has ceased; this effect, if regarded as undesirable, can be minimized by any one of a number of expedients.

PUMPS AND MOTORS

Several makes of centrifugal pumps with ½-inch outlets are in use, which will deliver solution at a rate of 20 liters per minute against a 6-foot head, when directly connected with motors of one-sixth horse-

power and 1,450 revolutions per minute. A turbine pump, which has a somewhat higher efficiency, is also successfully employed. The centrifugal pumps used for the operation of the deep cultures illustrated in figure 3 are regarded as especially satisfactory. They have ½-inch outlets, and are connected with an induction-repulsion motor of one-fourth horsepower and 1,450 revolutions per minute. These pumps have a discharge of 20 liters per minute against a 20-foot head, and deliver in the order of 40 liters per minute through plumbing arranged as shown in figure 3. The discharge from each pump is carried through a ¾-inch pipe. None of the pumps in use are self-priming; accordingly, it has been the custom to mount them outside the reservoirs, about 15 inches below the normal level of the solution. In the work at Rubidoux Laboratory, solutions are returned to volume each day, and the loss in any one day is never sufficient to drain the pumps. The plumbing of each unit is so arranged that new culture solutions can be circulated for mixing before being applied to the beds.

Consideration has been given to the necessity for using pumps made of special alloys to prevent or reduce corrosion. The abrasion factor introduced by the sand is largely avoided by using care in establishing the coarse sand and gravel below the fine quartz sand. One manufacturer recommended, as satisfactory from the corrosion standpoint, a chrome-nickel-alloy steel, and pointed out that this metal, referred to as one of the 18–8 metals, would offer good resistance to abrasion, being comparable in this respect to cast steel. One of the centrifugal pumps has a cast-iron impeller and the others have bronze impellers; all have given satisfactory service during the first year of use, without material evidence of corrosion.

A sentinel-type breaker switch is installed adjacent to each motor pump, permitting independent operation and providing protection to the equipment.

The motor pumps, according to their type and the favorableness of the quotation, cost from $20 to $50 each, complete with base.

EQUIPMENT FOR AUTOMATIC OPERATION

The automatic operation of the motor pumps at the desired hours and for the desired durations may be effected in a number of ways. Two systems whereby the operation of the motors was limited by light-sensitive elements to the hours of substantial transpiration have been tried and discarded. Both proved to be subject to failure and required attention. The equipment described herein is now employed and is regarded as satisfactory. There is also described a simpler equipment that the writer believes might be suitable for many experimental purposes.

The equipment in use is comprised of (1) a time switch, (2) an auxiliary duration timer, (3) a small interposed auxiliary contactor, (4) a 75-ampere triple-pole magnetic contactor, and (5) a selector switch to permit operation of equipment automatically from the timer or by hand.

(1) *Time switch* (G. E. type T–15 time switch).—This piece has an electric clock with a 24-hour dial. The dial carries a series of movable "on" and "off" riders which actuate a mercury-to-mercury switch. The riders may be set to operate the pump equipment at intervals of

1 hour or longer. The minimum period between an "on" and "off" rider is one-half hour.

(*2*) *Auxiliary timer* (G. E. type TSA-10 timer).—This piece controls the duration of each operation of the motor pumps. The timing element consists of a small synchronous motor. By means of an electromagnetic clutch, the motor drives a pointer across a scale graduated in minutes. An adjustable arm with contact points is set on the scale for the desired time interval of operation. When the moving arm reaches the adjustable arm the contacts are opened, and shut down the motor pumps. The contacts on the adjustable arm are normally closed and are rated to carry 1 ampere at 115 or 230 volts.

(*3*) *Interposed auxiliary contactor* (G. E. CR 2810–1265).—The contacts of this piece are normally open. Its use is necessitated by the fact that the main contacts of the auxiliary timer (2) are limited to 1 ampere, whereas the operating coil of the magnetic contactor (4) that controls the pump motors will take an operating-coil inrush current of considerably more than 1 ampere. The operating coil of this auxiliary contactor requires only a fraction of an ampere. The current passing though the contacts of the auxiliary timer (2) energizes the coil of this interposed contactor, closing its contacts and thereby completing the secondary circuit through the coil of the magnetic switch (4).

(*4*) *Seventy-five-ampere triple-pole magnetic contactor.*—This contactor will carry the load represented by upward to 30 motors of one-sixth horsepower each. Either 110- or 220-volt single-phase motors may be operated from this three-pole magnetic switch by connecting them across the appropriate lines. The 110-volt current is used in the operating coil and in pieces 1, 2, and 3.

(*5*) *Selector switch* (G. E. Type CR 2960–SY103A).—This switch is wired in with the other devices so that the automatic time equipment is inactive when the switch arm is in the "hand" position; when in the "automatic" position, operation of the pump motors is entirely dependent on the timing-control circuit, and when in the "off" position the motors are shut down and are not affected by the operation of the timing equipment. The piece may be dispensed with by installing a snap switch across the lines from the contacts of the interposed contactor (3) to the magnetic contactor (4). Either arrangement permits one to run the motors independently of the timing device when solutions are to be mixed or the reservoirs emptied.

The external wiring diagram for connecting these pieces is simple and can be readily sketched by a manufacturer's representative. The external sketch, however, has little meaning except in conjunction with a study of the internal wiring diagrams of the several pieces which become somewhat complex and are not easily reproduced.

Briefly stated, one operation carries through as follows: An "on" rider on the time clock (1) closes the mercury-to-mercury switch and a 115-volt line current flows through the normally closed contacts of the auxiliary duration timer (2), the clock motor of which is started, and on through the coil of the normally open interposed auxiliary contactor (3). The contacts of the latter close, completing a second 115-volt circuit through the operating coil of the large 75-ampere magnetic switch (4), which contactor closes, starting the pumps. The pointer of the auxiliary timer (2) moves on by clock and separates its

contacts at the end of the desired duration period. The breaking of this circuit deenergizes the coil of the interposed auxiliary contactor (3), allowing its contacts to open, which in turn breaks the circuit that has held the magnetic contactor (4) closed. The pumps are stopped at this point, but the cycle of the timing mechanism is not yet complete. When an "off" rider on the time switch (1) is reached, its mercury switch is opened. The opening of this switch automatically resets the auxiliary duration timer (2) for the next action.

The above control apparatus can be installed at a cost of about $100.

For some purposes the equipment for controlling pump operations may be greatly simplified by employing a time switch (G. E. type T–27) that has recently become available. As differing from the time switch (1), which has been described, an "off" rider of the newer type may be set to follow an "on" rider as closely as desired, but the minimum interval between "on" riders on the 24-hour dial is 1 hour and 45 minutes. The duration period cannot be adjusted as closely with these riders as it can be with the auxiliary timer described under (2). This timer employs, in place of the mercury-to-mercury switch, silver-button contacts capable of carrying a connected starting load of 40 amperes. A limited number of pump motors, accordingly, could be connected directly to the timer without accessory apparatus. Either with or without the magnetic switch, the time interval between operations can be reduced to 53 minutes by using two of these timers connected in parallel. The two timers, by appropriately setting the "on" riders, would alternate in operating the motor-pump or magnetic-switch circuits. The list price of this timer is about $20.

OPERATION

SELECTION OF SAND

Some importance is to be attached to the selection of quartz sand with particle sizes best suited to culture uses. If the sand is too coarse little water is held against gravity, whereas if the sand is too fine drainage is slow. Particularly undesirable is sand with particle sizes so mixed that little void remains when the sand has settled. Although this matter needs further study, it appears probable that no difficulty would be experienced with any sand held by a 100-mesh screen after passing a 40-mesh screen. A very satisfactory beach sand, taken at the foot of the Torrey Pines grade in southern California, had by weight 2 percent of 20–40-mesh particles, 25 percent of 40–60, 54 percent of 60–80, 13 percent of 80–100, and 6 percent of particles that passed a 100-mesh screen.

IRON SUPPLY

Experience at this laboratory has indicated that 0.1 percent of magnetite mixed with quartz sand makes unnecessary the use of soluble iron in the culture solutions maintained on the acid side of neutrality. A number of crop plants obtain sufficient iron from magnetite when the pH value is as high as 8. Pyrites may also be used as a source of iron if the medium is alkaline; on the acid side of neutrality, pyrites sometimes causes injury.[3] These data support the conclusion that iron may be obtained by plants from some water-

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[3] EATON, F. M., BLAIR, G. Y., and WILCOX, L. V. [Unpublished data.]

insoluble minerals by absorption across the particle-root interface, rather than by absorption from the culture or soil solution. The beach sand previously referred to in the present paper contains, in addition to constituents other than quartz, water-insoluble iron that is available to plants. Corn plants, which have a relatively high iron requirement, when grown in this sand showed no evidence of chlorosis if supplied with nutrient solutions maintained at pH values 5 to 6, 8, and 9.

CONTROL OF ORGANISMS

Free-living and pathogenic organisms tend to develop in sand cultures, particularly when the surface of the sand is not protected by gravel or metal plates to repress the growth of algae. Such an effect may become pronounced after several successive crops. It has, accordingly, become the writer's custom to apply a 0.5- to 1-percent solution of formaldehyde between crops. This solution is circulated by the pumps four or five times a day for several days, and is then washed out by several changes of water. The design of the equipment is such that steam or hot water can likewise be used for sterilization.

SALT ACCUMULATION AND DAMPING-OFF

Some minor evidence of accumulation of salts of low solubility on plant stems has been experienced when the concentrations of chlorides and sulphates have been made high in plant-tolerance studies. This has not been observed in the control beds, where only the usual nutrient solutions were used. The fact that solutions are applied at frequent intervals to a depth of 1 inch over the surface of the sand and then quickly drained away is doubtless largely responsible for general absence of salt incrustations. The lower portions of the stems, up which salt might creep from the sand, are submerged by each application of solution. The metal plates and gravel used to check algae growth serve likewise to reduce evaporation rates near the surface of the sand.

In the early seedling stages, when plants are most subject to damping-off, they transpire very little water. During this period root development is relatively rapid and it is not necessary to circulate the culture solutions, with the attendant wetting of the surface of the sand, since sufficient water and nutrients are supplied by the culture solution retained by the sand against drainage.

PRACTICAL SUGGESTIONS

USE OF SAND CULTURES IN HOTHOUSES

In a previous publication [4] the suggestion was offered that sand cultures might have practical advantages over soils in hothouse culture. Sand cultures are now being used commercially to a limited extent. In many cases plants supported by nutrient solutions in sand cultures have developed more rapidly and have been more fruitful than those grown in soils under otherwise similar conditions. Practical considerations incline the writer to the view that the usefulness of sand cultures is in no way limited to nutritional studies. The idea of maintaining the hydrogen-ion concentration and the concentrations and proportions of nutrients at levels best suited to the varied

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[4] EATON, F. M. See footnote 2.

requirements of plants grown in hothouses is an inviting one, and there is likewise to be taken into account the ease with which soil organisms may be controlled.

Sand beds of the type shown in figure 2 are regarded as being well-adapted to greenhouse use. If judicious use is made of thermostats, culture temperatures may be closely controlled by installing steam pipes or electrical heating cable in the sand. The temperature of the culture solutions can likewise be controlled.

Plants may be removed from sand cultures for transplanting to the field or pots with a minimum of injury to the root systems.

COMPOSITION OF CULTURE SOLUTIONS

The following material on the composition of nutrient solutions will be wholly familiar to students of plant nutrition. It is offered for the convenience of those whose interests are primarily in the propagation and culture of plants.

Plants will make a creditable growth when supplied with culture solutions of markedly different composition. A highly satisfactory culture solution for some kinds of plants may be poorly suited to others, and a solution that is superior during one season of the year may not be the most desirable in another season, since climatic conditions, as well as length of day, influence the nutrition of plants. During recent years there has been a tendency toward the use of solutions that are less concentrated than many of those employed by the earlier investigators.

The composition of two culture solutions is shown in table 1. The first of these is a satisfactory and extensively used nutrient popularly known as Hoagland's solution. The second solution, is one that has been used effectively in nutritional studies in sand cultures at the Rubidoux Laboratory.

TABLE 1.—*Composition of 2 nutrient solutions*

Solution and constituents	Millimoles per liter	Grams per 100 liters
Hoagland's solution:		
Calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$	5	[1] 118 (91)
Potassium nitrate, KNO_3	5	51
Magnesium sulphate, $MgSO_4 \cdot 7H_2O$	2	49
Potassium acid phosphate, KH_2PO_4	1	14
A Rubidoux Laboratory solution:		
Calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$	4	[1] 94 (72)
Potassium nitrate, KNO_3	3	30
Ammonium sulphate $(NH_4)_2SO_4$	2	27
Magnesium sulphate, $MgSO_4 \cdot 7H_2O$	2	49
Potassium acid phosphate, KH_2PO_4	.2	3

[1] Weights in parentheses are for granular calcium nitrate with 15.5 percent nitrogen.

Iron must be made available to plants supported by nutrient solutions. Mention has been made of the suitability of certain water-insoluble iron minerals. Iron citrate can be added to culture solutions at the rate of 1 cc of a 0.5-percent solution per liter of nutrient each several days or sufficiently often to maintain the plants in good condition. Like amounts of specially prepared iron humate,[5] iron tartrate, or ferrous sulphate are also effective.

[5] HORNER, C. K., BURK, D., and HOOVER, S. R. PREPARATION OF HUMATE IRON AND OTHER HUMATE METALS. Plant Physiol. 9: 663–669. 1934.

In addition to iron and the elements of the base nutrient solution, plants require small amounts of certain other elements. Boron, manganese, and zinc should be introduced. For most purposes 1 p. p. m. of boron, 0.2 p. p. m. of manganese, and 0.1 p. p. m. of zinc will prove satisfactory. To approximate these concentrations in 100 liters of solution, add 0.6 g of boric acid (H_3BO_3), 0.1 g of manganese chloride ($MnCl_2.4H_2O$), and 0.04 g of zinc sulphate ($ZnSO_4.7H_2O$). It is not implied that other elements in small quantities are not beneficial, but the need of particular ones in addition to the amounts introduced as impurities with the principal salts has not yet been clearly defined. Many elements are contained in quartz sand, and they are likewise introduced if tap water is used in the preparation of solutions. So-called A-to-Z mixtures of miscellaneous elements, in very small amounts, are sometimes added to culture solutions, as are extracts of manure and soils.

The phosphate in culture solutions tends to precipitate the iron. Recent work by Olsen[6] shows further that absorbed iron may be precipitated and rendered inactive within the plant when the phosphate content of the nutrient is high. Hoagland's solution is often made up with only a tenth as much potassium phosphate as that given in the formula, and the phosphate content of the second solution is purposely made low for this reason.

For general-purpose culture solutions it is desirable to introduce a part of the nitrogen as ammonium ion. Unless tap water is used in making up the nutrient, 2 millimoles per liter of sodium chloride, NaCl (12 grams per 100 liters), should be added. The growth of some plants, tomatoes for example, will be substantially improved as a result of introducing this salt.

Tiedjens[7], working with apples and tomatoes, has reported that nitrate ion was assimilated most satisfactorily when absorbed from an acid solution of approximately pH 4.0 whereas ammonium ion was assimilated most satisfactorily when absorbed from nutrient solutions having pH values of 5.0 to 6.5, varying somewhat with different varieties. In general, ammonium and nitrate salts produced equally good growth provided their limitations were recognized. Trelease and Trelease[8] have proposed the use of solutions balanced with respect to NO_3/NH_4 ions to maintain stable hydrogen-ion concentrations.

If culture solutions are extensively employed, consideration can properly be given to the use of technical and commercial grades of chemicals, but some caution must be employed in their selection. The synthetic commercial fertilizers, such as ammonium sulphate and granular calcium nitrate, are usually relatively pure.

The frequency with which old solutions must be discarded and new ones substituted is always an individual problem, related, of course, to the volume of the supply and the size of the plants. Potassium, ammonium, and nitrate ions tend to become deficient in culture solutions more rapidly than some others. These and other constituents can be replaced as they are taken up by plants if laboratory facilities for their quantitative determination are available; otherwise it is desirable to substitute new solutions at frequent intervals.

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[7] TIEDJENS, V. A. FACTORS AFFECTING ASSIMILATION OF AMMONIUM AND NITRATE ION, PARTICULARLY IN TOMATO AND APPLE. Plant Physiol. 9: 31–57, illus. 1934.
[8] TRELEASE, S. F., and TRELEASE, H. M. PHYSIOLOGICALLY BALANCED CULTURE SOLUTIONS WITH STABLE HYDROGEN-ION CONCENTRATION. Science (n. s.) 78: 438–439. 1933.

DISCUSSION

The electrical and mechanical features of the sand cultures here described have required little attention other than an occasional greasing and oiling of the pumps and motors. The unattended operation of the equipment on Saturday afternoons and Sundays has been entirely satisfactory. If much phosphate is used in culture solutions, it appears possible that calcium phosphate might accumulate in the pumps to such an extent that they would require cleaning.

The best quartz sand contains impurities that are not removed by digestion in strong acid, and for that reason water-culture technique is essential to some research. In water-culture technique practically all of the advantages are in favor of the large-vessel aerated type. Many workers have found flowing cultures inadequate as a means of maintaining uniform concentrations, except at the expense of wasting large volumes of solution, because the diverse conditions that exist between day and night and at the successive stages of plant development defy corresponding adjustments in the rate of supply of new solution. Problems concerned with seedling germination, aeration, iron supply, plant supports, and with less tangible matters attend the use of all forms of water culture. These difficulties are minimized in sand cultures, the use of which limits the care that must be devoted directly to plants during their growth to such operations as thinning, spraying for pests, and staking to prevent wind injury. The labor-saving advantages, however, may be regarded as somewhat incidental to the opportunity afforded, by the equipment here described, for closely controlling and defining the concentrations of solution constituents in sand cultures. In the majority of investigations, the traces of miscellaneous elements introduced by the quartz sand may be regarded as advantageous.

SUMMARY

The improved automatically operated sand-culture equipment described herein is designed to provide (1) the advantages of large-vessel, or flowing-type, water cultures with regard to the maintenance of solution concentration and (2) the numerous advantages of sand cultures, such as aeration, iron supply, seedling germination, root environment, and elimination of plant supports.

The solution is applied by motor pumps controlled by a time clock to the surface of free-draining sand cultures at hourly or other selected intervals. The displaced solution returns by gravity to the supply reservoir.

Equipment in use at the Rubidoux Laboratory is described, and suggestions are offered on the construction of sand beds and solution reservoirs, on types of sand, iron supply from water-insoluble minerals, control of troublesome organisms in the sand, and the applicability of the method to hothouse culture.

A brief account of the composition of certain culture solutions is given for the convenience of those interested in the propagation and culture of plants.

THE DEVELOPMENT OF THE WHEAT SPIKE[1]

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INTRODUCTION

All parts of a wheat stem arise from its growing point. In the early stages of growth only leaves are produced, while in later stages the spike and its parts are produced. The time of the transition from the early, or vegetative stage, to the later, or reproductive stage, of development has a bearing upon the earliness and lateness of heading of wheat varieties. The effect of low-temperature germination (vernalization) in bringing about earlier heading in certain varieties of wheat, is related to the effect of such treatment upon earliness of spike differentiation.

Some studies of the development of the wheat spike have been published, but more attention has been given to the development of the kernel than to that of the spike. An early contribution to a better understanding of the development of the wheat plant was made by Carruthers (2).[2] Jensen (4) has cited a number of publications dealing with certain phases of morphological development of the wheat plant, and he has described and illustrated various phases of spike and flower development. More recently Percival (8), Kiesselbach and Sprague (5), and Noguchi (7) have described and presented line drawings of certain phases of spike and spikelet development. McCall (6) has studied the developmental anatomy and morphology of the embryo and seedling of wheat.

This is the second of a series of articles dealing with the ontogeny of the inflorescence of small grains. The first article dealt with the development of the barley spike (1). The present article describes briefly, and illustrates with photomicrographs, the principal stages in the development of the growing point, the spike, and the spikelet of the wheat (*Triticum aestivum* L.) plant.

MATERIALS AND METHODS

The methods used in handling the dissected material and in taking most of the photomicrographs were the same as those described in the earlier paper on barley (1). Some of the photographs were taken with a 32-mm Micro Tessar lens.

The plants from which the growing points and spikes were dissected were grown in the field. Collections were made from time to time during the growing season. Most of the plants used were Purkof, a beardless, semihard, red winter variety of wheat.

DESCRIPTION OF SPIKE DEVELOPMENT

The growing point of a head-bearing wheat stem in its growth from germination to pollination passes through two stages of development.

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The first is the vegetative stage, which extends from seeding in the fall through the winter to late March or early April. In this stage the internodes of the stem remain short and only leaf initials are produced by the growing point. The growing point elongates but remains nearly smooth in outline. This is also the stage of active tiller development. The beginning of the second, or reproductive stage, is indicated by the appearance of double ridges, the upper member of which develops into the spikelet initial. During the reproductive stage certain of the internodes of the stem elongate and the spikelet parts differentiate and increase in size. The vegetative stage is shown in plate 1, *A*, *B*, and *C*, and the reproductive stage in plate 1, *D*, to plate 2, *F*, inclusive. Thus, it is seen that the developmental stages of the wheat plant are very similar to those of barley, already described (*1*).

The shoot of the embryo of a wheat kernel has only a few parts. They are the coleoptile, the first, second, third, and fourth leaf initials, the growing point, and a tiller bud in the axil of the coleoptile.

The parts of the shoot and their arrangement in the embryo can be observed in seeds which have been soaked for about 24 hours. The coleoptile completely encloses the leaf initials and the growing point. In the axil of the coleoptile, opposite the scutellum, the tiller bud with its first (prophyllum) and second leaf initials, and the growing point can be located. The first leaf if slightly shorter than the coleoptile, which also encloses the growing point, and its fold is on the side next to the scutellum. The second and third leaf initials only partly enclose the growing point. The fold of the second leaf initial is on the side opposite the scutellum, and the fold of the third leaf is on the same side as the first leaf. Across the growing point on the side next to the scutellum is a ridge, which is the beginning of the fourth leaf. A more detailed description of all the structures mentioned has been given by Percival (*6*).

By the time a wheat plant has two leaves the sixth leaf initial can be easily seen (pl. 1, *A*, l_6). The rest of the leaves range in size from the fifth leaf initial, which nearly encloses the growing point, up to the fully matured first leaf. The growing point at this time is short and hemispherical (pl. 1, *A*, *g*).

Leaves begin their development as lateral, alternate ridges (pl. 1, *B*, *x*) beneath the apex of the growing point. The leaf initials arise in acropetal order, become larger on passing farther from the tip, and nearly encircle the growing point. Each ridge is most prominent on the side opposite the leaf initial just beneath it. The more prominent side of the ridge forms the apex of the young leaf (pl. 1, *A*, l_5, and pl. 2, *C*, *l*), and this is the oldest portion of the leaf since grass leaves elongate by basal growth.

Each leaf in succession grows up over the growing point or the spike and inside of the preceding leaf. A leaf partly enclosing the spike is shown in plate 2, *C*. This leaf was removed and a photomicrograph was taken to show the spike and the size of the succeeding leaves (pl. 2, *D*, *l* and *l'*). The next leaf (pl. 2, *D*, *l*) has just started to elongate. The two leaf initials appear to be the last initials formed on this stem.

The vegetative stage of stem development in winter wheat is shown clearly in plate 1, *B*. This growing point was removed on October 17 from one of the largest stems of a volunteer wheat plant 50 to 60 days

old. The growing point is short. Ten leaves were removed to expose the growing point and there still remained four well-developed leaf initials at the base and four ridgelike leaf initials farther up on the growing point.

When active growth starts in the spring the growing point begins to elongate in preparation for spike differentiation (pl. 1, *C, g*). Leaf initials can be seen at the base of the growing point and there is some indication of ridges higher up, but in contrast with the growing point in plate 1, *B, g* it is relatively smooth in outline. When this plant was sampled on March 28, the internodes of the stem had not begun to elongate.

After the elongation of the growing point the first indication of spikelet development is the appearance of double ridges (pl. 1, *D, x*, and *E, x*). The lower or smaller ridge of the pair can be seen until the upper member of the pair bcomes large enough to cover it. The upper ridge elongates and attains considerable size and prominence before the spikelet structures begin to differentiate (pl. 1, *F, si*).

It is of interest to note the changes in the shape of the spike and the difference in the size of the spikelet initials in either direction from the middle (pl. 1, *F*). The shape of the spike at this stage is lanceolate. The spikelet initials show the greatest elongation in the middle of the spike (pl. 1, *F, si*). From the middle toward the base and tip of the spike, the spikelet initials are successively shorter, but the tip spikelets are not as long as those at the base.

Differentiation of each of the spikelet structures begins first in those middle spikelets which show the greatest elongation (pl. 1, *F, si*), and in succession in other spikelets of the head basipetally and acropetally. The terminal spikelet (pl. 1, *F, t*) is the last to differentiate. This sequence of development among the spikelets is maintained throughout the development of the spike, including anthesis and kernel development. In the mature spike the order of the average kernel weight of a spikelet is the same as the order of spikelet differentiation, viz, the heaviest kernels are in the spikelets in the middle of the spike where differentiation first begins, and they decrease in weight from this point toward the base and the tip.

The empty glumes are the first spikelet structures to differentiate (pl. 1, *G, c*). They appear as transverse ridges on both sides of the spikelet initial. A little later other ridges appear just above and parallel to the first. The second ridges become the lemmas of the first and second flowers (pl. 1, *H, gl*). Flower parts develop from the hemispherically shaped meristem above the lemma initial (pl. 1, *H, r*).

Anther differentiation begins in the basal flowers of the spikelets located about halfway up the spike. Three slight elevations appear upon the meristem above the lemma initial and these soon enlarge (pl. 1, *I, an*).

A wheat spikelet is many-flowered. This is shown in plate 2, *A*, *fl*₁ to *fl*₅ and *E*. The first-mentioned figure represents a spike of a bearded wheat, sampled May 2, and chosen to give an idea of the appearance of the spike and the arrangement of the flowers in the spikelets. Flower position in the spikelet is better shown by the ventral (left) and dorsal (right) view of the two wheat spikelets in plate 2, *E*. Each spikelet has six flowers arranged alternately and attached to the short rachilla which is hidden by the flowers. Flowers

1 to 3, inclusive, show anthers. Anther differentiation has just begun in flower 4, but none can be seen in flowers 5 and 6. The beginning of awn development can be seen in plate 2, *E, a.*

As mentioned above, flower differentiation and development in the wheat spikelet proceeds from the base upward in acropetal order. The empty glumes on both sides of the base of the spikelet differentiate first. Soon the lemmas of the first and second flowers appear, and the meristem from which the stamen and pistil differentiate becomes prominent and hemispherical. Differentiation in the third, fourth, fifth, and sixth flowers follows in the order named. As shown in plate 2, *E*, the stages of flower development range from those at the base with all parts fully differentiated to those at the tip with the lemma just differentiating.

By the time the last internode (peduncle) of the stem begins to elongate the glumes have enclosed the anthers and pistil (pl. 2, *B*). The awns of the awned variety have considerable length and show the presence of barbs.

It should be noted in plate 1, *H, t,* and *I, t,* that the wheat spike terminates in an apical spikelet, and hence may be classed as a determinate inflorescence. Before the spikelet and flower parts begin to differentiate (pl. 1, *F*) the number of spikelets that a wheat spike will have, has already been determined.

Only one stage of pistil development is shown (pl. 2, *F*). A portion of the flower has been removed to show the pistil. At this stage the ovary and styles are well formed but the stigmatic branches have not yet appeared.

The plants in plate 1, whether or not grown the same year, show the various stages in the development of the growing point and spike as then would be found at the approximate time of year indicated, under the climatic conditions prevailing at Urbana, Ill. The plant shown in plate 1, *A*, was sampled on October 19, 1935, and that in plate 1, *B*, on October 17, 1935. Plants shown in plate 1, *C* and *D*, were sampled on March 28 and April 2, 1935, respectively. The dates of sampling the other plants shown in plate 1 were as follows: *E*, April 10; *F*, April 21; *G*, April 28; *H*, April 28; and *I*, May 5 in 1934.

DISCUSSION

The probable morphological significance of the lower of the pair of ridges, which mark the transition of the growing point of wheat and barley (*1*) from the vegetative to the reproductive phase, should be pointed out. It is clear from observation that all of the spikelet

PLATE 2

parts differentiate from the upper of the pair, but the lower ridge is soon covered by the growth of the spikelet, and hence, its history can only be inferred. An explanation suggested by the writer for barley was that the lower of the pair becomes the internode of the rachis. This explanation was not correct, although, as will be pointed out later, the ridge does merge with the tissues of the node.

In a study of the influence of temperature during germination on the subsequent development of certain cereals, Purvis (*9*) noted double ridges on the growing point. She pointed out that the double ridges suggested a leaf and its axillary bud. This suggestion is accepted as correct. Hitchcock (*3*) states: "The spikelet is, therefore, a reduced modified shoot in which the rachilla is a stem bearing at each node a reduced leaf (bract)." Since lateral shoots of grasses originate from the main shoot in the axil of the leaf sheath, the view that the upper ridge from which the spikelet differentiates is an axillary bud, and the lower ridge the rudiment of a leaf, is in harmony with the morphology. The mature spike of wheat as well as of barley has a rudimentary leaf and a spikelet in its axil at the first node and a distinct ridge at the next higher node. At the other nodes of the mature spike the ridge cannot be seen but merges with the nodal tissue.

Since a plant cannot anticipate its environment it must be able to make adjustments to growing conditions. Wheat and barley (*1*) spikes present interesting contrasts in their manner of making adjustments to the environment. Two-rowed barley has only one, and six-rowed barley has only three, single-flowered spikelets that may become fertile, at each joint of the rachis. However, the barley spike is an indeterminate inflorescence and any variation in the number of flowers in a spike can take place at the tip of the spike. The wheat spike, on the other hand, is a determinate inflorescence which terminates in an apical spikelet, so that as soon as the spikelets differentiate their number is fixed. While only one spikelet develops at each node of the rachis, each wheat spikelet has six or more flowers. In most varieties of wheat the two basal flowers of each spikelet are fertile, but under good growing conditions more flowers may become fertile. Thus in barley any variation in the number of flowers in a spike takes place at the tip of the spike, while in wheat any variation in the number of flowers in a spike takes place within the spikelets.

If good conditions for growth, are followed by poor conditions adjustments take place in an orderly sequence. On the spike the last-formed spikelets are the first to cease development and become abortive. Thus abortive spikelets in both wheat and barley are normally found at the base and tip of the spike. Likewise in multiflowered spikelets the last-formed, youngest flowers are the first to suffer under adverse growing conditions.

EXPLANATORY LEGEND FOR PLATE 2

A.—Young spike of wheat showing development and arrangement of flowers in the spikelets: fl_1 to fl_6, Basal, or first flower, to fifth flower, respectively; *an*, anthers. ×30.
B.—Spikes of a bearded and a beardless variety of wheat, at the beginning of the elongation of the peduncle. ×2.5.
C.—A spike of wheat showing how leaves grow up over the spike: *l*, Leaf initial; *s*, spike. ×44.
D.—Same spike as in plate 2, *C*, but with large leaf removed to show size of next smaller leaves and the stage of spike development: *l* and *l′*, Leaf initials. ×44.
E.—Ventral (left) and dorsal (right) views of dissected spikelets of wheat: *e*, Empty glumes; *gl*, lemma; *a*, awns; fl_1 to fl_6, flowers. ×20.
F.—A flower with a portion removed to show a stage of pistil development: *p*, Pistil; *an*, anthers. ×20

SUMMARY

A study of the morphological development of the growing point and spike of wheat was made by dissecting the growing points and spikes from stems at different stages of development. Photomicrographs of the principal stages are presented.

In the resting stage of the seed the stem portion of the wheat embryo consists of the following parts: Coleoptile, first, second, third, and fourth leaf initials, the coleoptile tiller bud, and the growing point. Each of the parts mentioned can be dissected from a seed that has been soaked in water for a few hours.

Leaf initials appear as alternate ridges which nearly encircle the growing point. The ridges are more prominent on the side opposite the leaf initial just below or above it. The prominent portion forms the apex of the young leaf. Leaves elongate by basal growth and grow up inside the preceding leaf.

During the fall, winter, and early spring the growing point remains in the vegetative stage. In the fall the growing point produces only leaf initials, while in early spring, in addition to the production of leaf initials, the growing point elongates. The beginning of the reproductive stage is marked by the apperance of double ridges the upper of which produces the spikelet and its parts. During the reproductive stage the spikelets and spikelet parts differentiate and increase in size.

Spikelet differentiation begins in the middle of the spike and proceeds toward the base and the tip of the spike. Likewise, each of the spikelet parts in the sequence of spikelet development appears first in the spikelets in the middle of the spike.

Within the spikelet, differentiation begins at the base of the spikelet and proceeds upward. Within the flower, the sequence of differentiation of its members proceeds from the outside inward. The pistil and its parts are the last to differentiate.

Spikelet parts differentiate in the following order: Empty glumes, and flowers 1, 2, 3, etc. Within the flower the order of differentiation is: Lemma, anthers, palea, and pistil. The sequence of the pistil parts is: Ovary, styles, and stigmas.

The wheat spike is a determinate inflorescence. It terminates in an apical spikelet placed at a right angle to the plane of the rest of the spikelets. When the spikelets differentiate the number is fixed but adjustment to growth conditions may be made in the number of fertile flowers in a spikelet.

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EFFECT OF PACKING-HOUSE BYPRODUCTS, IN THE DIET OF CHICKENS, ON THE PRODUCTION AND HATCHABILITY OF EGGS [1]

By HARRY W. TITUS, *biological chemist;* T. C. BYERLY, *physiologist;* N. R. ELLIS, *chemist;* and R. B. NESTLER, *junior biologist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In an earlier paper Byerly, Titus, and Ellis [2] stated that some lots of meat meal are deficient in some substance that is necessary for the production of eggs capable of supporting embryonic life during the second week of incubation.

In view of the importance of the byproducts of the packing house as protein supplements in the feeding of poultry, it was decided to study further the effect of these materials on the production and hatchability of eggs. This paper presents the pertinent data on the subject obtained by the writers from the fall of 1929 to the fall of 1934, at the Animal Husbandry Experiment Farm of the National Agricultural Research Center, Beltsville, Md.

EXPERIMENTAL METHODS AND MATERIAL

The experiments involved feeding tests with Single-Comb Rhode Island Red pullets. The duration of each experiment was twelve 4-week periods, or 336 days. During each 4-week period some or all of the eggs laid were incubated. The pullets were distributed among the pens so that each contained from 15 to 40 birds. They were selected and distributed in a manner to insure uniform lots with respect to size, weight, and age. These pens received diets containing various protein supplements to be appraised experimentally. The birds were comfortably housed; straw litter was used; feeding was conducted in a uniform manner; and accurate egg records were insured by trap-nesting. Further details regarding selection of the birds, their housing, feeding, and management are given in a paper by Byerly, Titus, and Ellis.[3]

The present experiments dealt chiefly with the effects of packing-house byproducts on production and hatchability of eggs. Data also were obtained on the influence of the various diets on live weight, weight of eggs, feed consumption, and embryonic mortality. The study involved several different methods of approach. One of these, that of the first year (1929–30), was a comparison of a typical packing-house byproduct, desiccated meat meal, with (1) a diet containing no protein supplement and (2) a diet containing a meat-fish-milk supplement, already known to be excellent in its effect on egg production and hatchability. The diet containing the meat-fish-milk

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supplement, which is not a packing-house product, may be considered as a positive control and the diet containing no protein supplement as a negative control. Further comparisons of the positive and negative control diets were made the 2 following years. During the fourth year the experiment dealt with comparisons, for poultry feeding, of other packing-house byproducts as prepared by ordinary commercial methods. During the fifth year (1933–34) the test dealt principally with all-beef scrap prepared by methods involving close control over periods and temperatures of cooking.

Throughout the entire period of the experiments the diets contained an adequate supply of necessary mineral elements. In the first year's work a fixed quantity of mineral mixture was incorporated in each diet, whereas during the last 4 years all diets contained a fixed quantity of anhydrous sodium sulphate and common salt (0.5 percent of each) but different quantities of ground limestone and steamed bonemeal. In 1930–31, the quantities of the last two materials were so adjusted as to keep the phosphorus content between 0.5 and 0.9 percent and have a calcium-phosphorus ratio of 4:1. In each of the last 3 years, 1931–34, the adjustment was so made that all the diets contained approximately 1.2 percent of total phosphorus and 3.0 percent of total calcium.

BASAL DIETS

In compounding each of the diets studied a basal feed mixture was used, which consisted of the following:

	Parts by weight
Ground yellow corn	500
Wheat bran	245
Rolled oats	150
Alfalfa-leaf meal	55
Total	950

The basal diet, which contained no protein supplement, varied somewhat from year to year. Its composition is given in table 1.

The minor variations in the composition of the basal diet broadened the scope of the investigation in that the tests included slightly different diets of the type used by investigators and in commercial poultry establishments. During the course of a particular year, however, the basic conditions of the experiment and of environmental factors were uniform for all pens involved in the comparative studies. Thus all results within a year are closely comparable, whereas those among the different years are only approximately comparable.

TABLE 1.—*Variations from year to year in composition of the basal diet which contained no protein supplement*

Constituent	Parts by weight		
	First year	Second year	Third year
Basal feed mixture	95.0	93.6	89.9
Ground limestone	2.5	5.4	4.0
Steamed bonemeal	1.5	.0	5.1
Anhydrous sodium sulphate	.5	.5	.5
Common salt	.5	.5	.5
Total	100.0	100.0	100.0

<div align="center">THE PROTEIN SUPPLEMENTS</div>

Each of the other diets contained 20 parts, per 100 parts, of one of the packing-house byproducts used or of the meat-fish-milk protein supplement. The quantities of basal feed mixture, ground limestone, and steamed bonemeal were so adjusted that the diets contained the desired percentage of calcium and phosphorus.

In order to insure an adequate supply of vitamins A and D, 2 parts by weight of cod-liver oil were added to each 100 parts of the diets just before they were fed.

The protein supplements used in compounding the individual diets are described below. In 1932 and 1933 the Institute of American Meat Packers prepared a number of special products for use in this study. These are indicated by the letters "I. A. M. P." placed immediately after the name of the supplement.

Meat-fish-milk.—This supplement, which is not a packing-house byproduct but which was used in these experiments as a positive control, consisted of 40 percent of desiccated meat meal (described below), 35 percent of whitefish meal, and 25 percent of dried buttermilk. The whitefish meal was a commercial product prepared from the offal of the North Atlantic fishing industry by the vacuum-drying process. The dried buttermilk was the usual commercial product commonly used in feeding poultry.

Desiccated meat meal.—This is a special meat meal regularly prepared for the Bureau of Animal Industry, and is not available commercially. The material used in its preparation is the flesh of condemned carcasses of various kinds. It is processed in much the same manner as ordinary meat meals. It differs from the latter, however, in that it contains little bone, and its protein content is usually between 75 and 80 percent and its ash content usually less than 7.5 percent.

Ground, dried, lean meat (I. A. M. P.).—Only lean meat was used in making this supplement. It was a high-grade, ground, dried-meat product and was suitable for human consumption.

All-beef scrap no. 1 (I. A. M. P.).—This was a product such as is made for commercial purposes; it contained, however, no added high-protein materials such as blood meal and stick. The materials used in its preparation were: Carcasses, livers, spleens (known commercially as melts or milts), skulls (heads from which muscle tissue, skin, tongues, and brains were removed), beef rennets, tripe trimmings, hashed pecks (a packing-house term for finely chopped manyplies, the tissues of the third stomach of the ruminant), and beef-cutting scrap. No definite formula was used.

Meat-and-bone meal (55 percent protein) (I. A. M. P.).—This product was prepared from the meat-and-bone meal described below, with the addition of enough blood meal (about 25 percent) to bring the protein content to approximately 55 percent. The resulting product contained about 20 percent of blood meal.

Meat-and-bone meal containing no blood meal, stick, or other material added for raising the protein content (I. A. M. P.).—Skulls, livers, spleens, beef rennets, tripe trimmings, and hashed pecks were used in preparing this product.

Blood meal and stick (I. A. M. P.).—This consisted of a mixture of blood meal and stick in the proportions in which they commonly have been used in the manufacture of meat-and-bone meals of a relatively high protein content.

All-beef scrap no. 2 (I. A. M. P.).—This product was prepared from the same materials that were used in preparing all-beef scrap no. 1 (I. A. M. P.). In all-beef scrap no. 2, the following definite formula was used:

Percent

Carcasses	20
Livers	10
Spleens	10
Skulls	10
Beef rennets	15
Tripe trimmings	10
Hashed pecks	15
Beef-cutting scrap	10
Total	100

The materials were mixed and then cooked in a vacuum (−20 to −26 inches of mercury) for 4 hours at a maximum temperature of 178° F.

All-beef scrap no. 2A (I. A. M. P.).—This product was the same as all-beef scrap no. 2 except that the materials were cooked in a vacuum (−21 to −25 inches of mercury) for 6 hours at a maximum temperature of 178° F.

All-beef scrap no. 2B (I. A. M. P.).—This product was the same as all-beef scrap no. 2 (I. A. M. P.) except that the materials were cooked in a vacuum (−20 to −24 inches of mercury) for 16 hours at a maximum temperature of 178° F.

All-beef scrap no. 2C (I. A. M. P.).—This product also was the same as all-beef scrap no. 2 (I. A. M. P.) except that the materials were cooked at atmospheric pressure for 8 hours at a maximum temperature of 200° F.

Mixture of 80 percent of all-beef scrap no. 2 and 20 percent of steam-dried blood meal.

Mixture of 80 percent of all-beef scrap no. 2 and 20 percent of spray-dried blood meal.

Mixture of 80 percent of all-beef scrap no. 2, 10 percent of steam-dried blood meal, and 10 percent of liquid stick.

Mixture of 80 percent of all-beef scrap no. 2, 10 percent of spray-dried blood meal, and 10 percent of liquid stick.

A commercial meat-and-bone meal.—This product was purchased on the open market.

Liquid stick (I. A. M. P.).—Liquid stick is the concentrated liquor from the steam rendering of fatty animal tissue. It consists largely of water-soluble compounds, including many of the products resulting from the hydrolysis of protein. This byproduct is not used as such by the poultryman; nevertheless, it was studied because it is an ingredient of some of the meat byproducts that he uses.

The Institute of American Meat Packers supplied the two kinds of blood meal used.

METHOD OF DETERMINING THE VITAMIN G CONTENT OF THE PROTEIN SUPPLEMENTS USED IN 1933–34

The materials supplied by the Institute of American Meat Packers for use in the 1933–34 experiment included two types of blood meal—steam-dried and spray-dried—and four lots of all-beef scrap which had been manufactured under different, but carefully controlled, conditions. Accordingly, these materials were assayed for vitamin G in order to ascertain whether or not the method of drying, in the case of the blood meals, or the temperature and length of time used in processing, in the case of the all-beef scraps, had affected the vitamin G content.

The assays were made by means of the rat-growth method. The procedure followed was essentially the same as that used in earlier work reported by Ellis, Miller, Titus, and Byerly.[4] The basal diet fed to the rats consisted of 20 percent of alcohol-extracted casein, 65 percent of partially dextrinized cornstarch, 4 percent of mineral mixture, 1 percent of agar-agar, and 10 percent of lard. Vitamins A and D were supplied by cod-liver oil, which was fed separately. A Lloyd's reagent adsorbate prepared from an aqueous extract of rice polish was added as a source of vitamin B. This adsorbate was comparable in vitamin B potency to the international vitamin B standard. A control group of rats was fed the basal diet, and other groups were fed the same diet and were given each day weighed quantities of the packing-house byproducts. The gains in weight of the rats receiving the packing-house byproducts were corrected for the change in weight of the rats in the control group and were then used for computing the vitamin G content of the materials being assayed.

[4] ELLIS, N. R., MILLER, D., TITUS, H. W., and BYERLY, T. C. EFFECT OF DIET ON EGG COMPOSITION. III. THE RELATION OF DIET TO THE VITAMIN B AND VITAMIN G CONTENT OF EGGS, TOGETHER WITH OBSERVATIONS ON THE VITAMIN A CONTENT. Jour. Nutrition 6:243–262, illus. 1933.

EXPERIMENTAL RESULTS

The results of the vitamin G assays made on the packing-house byproducts used during the fifth year of the investigation are given in table 2. The lack of any significant difference in vitamin G content among the four lots of all-beef scrap indicates that the temperature used and the length of time the lots were cooked had no marked effect on the vitamin G content of this product. Furthermore, according to the data presented in this table, the liquid stick used was a fairly good source of vitamin G inasmuch as it contained approximately as much as the different lots of all-beef scrap. These data also indicate that the method of drying the blood meal had no marked effect on the vitamin G content of this product.

TABLE 2.—*Vitamin G content of packing-house byproducts used as protein supplements in 1933–34*

Product	Rats	Level fed daily	Average gain in weight during		Vitamin G per gram (approximate)
			8 weeks	1 week	
	Number	*Grams*	*Grams*	*Grams*	*Units*
All-beef scrap no. 2 (cooked 4 hours at maximum temperature of 178° F.)...	7	0.5	38.3±2.1	4.8	3
All-beef scrap no. 2A (cooked 6 hours at maximum temperature of 178° F.)...	7	.5	40.1±1.4	5.0	3
All-beef scrap no. 2B (cooked 16 hours at maximum temperature of 178° F.)...	7	.5	46.4±2.7	5.8	3
All-beef scrap no. 2C (cooked 8 hours at maximum temperature of 200° F.)...	7	.5	41.9±0.9	5.2	3
Liquid stick...	7	.5	37.9±3.5	4.7	3
Steam-dried blood meal...	7	.5	18.7±1.8	2.3	1.5
Spray-dried blood meal...	7	.5	16.9±2.5	2.1	1.5

The pertinent data obtained in the chicken-feeding experiments are summarized in table 3.

EGG PRODUCTION AND FEED CONSUMPTION

As a measure of egg production, it is customary to use the figure obtained by multiplying the total number of eggs produced by a bird, or a flock, by 100 and dividing the result by the corresponding number of bird days. This figure is referred to as the percentage of egg production. Thus, if each bird in a flock lays one egg per day during a given period, the egg production for that period is 100 percent. When mortality in the flock is low, this measure may be fairly reliable, but when mortality is heavy, it cannot be depended on.

A more dependable measure of egg production is to adjust the average number of eggs laid for the number of bird days by the method of covariance analysis and then multiply the adjusted average by 100 and divide the result by the average number of bird days. When this procedure is followed, it is possible to obtain also a good estimate of the standard error of the percentage of egg production and thus have a statistical measure of its reliability.

Because of the extensive use of the first method among investigators and to provide comparisons, the percentage of egg production of the birds on the different diets was calculated by both methods, and the results are given in table 3. The values obtained by the two methods agree rather well except in the case of the diets fed during 1932–33, when the mortality was heavy.

TABLE 3.—*Data on protein supplements, fed to Rhode Island Red pullets, in relation to production and hatchability of eggs, live weight, and feed consumption*

Protein supplement	Protein content of— Protein supplement	Protein content of— Diet	Pullets at beginning of experiment	Total bird days	Total bird days as percentage of possible number	Live weight of pullets Average initial	Live weight of pullets Average maximum	Live weight of pullets Average final	Feed consumed Per bird (336 days)	Feed consumed Per egg	Egg production 100× total eggs÷ total bird days	Egg production Adjusted by covariance analysis	Average egg weight	Second-week embryonic mortality	Hatchability of fertile eggs
	Percent	*Percent*	*Number*	*Number*	*Percent*	*Grams*	*Grams*	*Grams*	*Kilograms*	*Kilograms*	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
FIRST YEAR (1929–30)															
None		11.6	40	13,177	98.0	1,722	2,693	2,121	40	0.38	31.2	31.7±2.1	55.0	5.7	70.6±3.3
Meat+fish+milk	61.5	20.7	40	13,186	98.1	1,728	2,804	2,475	43	.30	42.7	43.0±2.1	58.2	5.8	72.7±1.9
Desiccated meat meal	72.4	23.8	40	12,891	95.9	1,770	2,757	2,466	44	.31	41.5	42.1±2.1	57.8	5.	70.1±2.7
SECOND YEAR (1930–31)															
None		11.6	25	7,832	93.2	2,034	2,350	1,978	36	0.34	31.5	31.4±1.9	53.4	18.1	52.2±6.2
Meat+fish+milk	59.3	20.7	15	4,947	98.2	1,644	2,432	2,272	42	.25	53.2	52.8±2.4	56.9	1.5	73.8±3.6
THIRD YEAR (1931–32)															
None		11.7	25	5,568	65.0	1,670	2,330	2,016	38	0.33	26.3	27.4±4.4	54.5	8.5	63.9±8.2
Meat+fish+milk	59.6	21.3	25	7,191	85.6	1,660	2,399	2,209	36	.29	35.0	34.2±4.4	55.8	2.1	68.1±3.3
FOURTH YEAR (1932–33)															
None		11.8	25	5,703	67.9	1,940	2,288	2,019	35	0.36	19.9	19.8±3.5	53.0	6.0	76.5±8.7
Meat+fish+milk	58.8	20.9	25	5,828	69.4	1,917	2,378	2,194	35	.34	24.6	23.1±3.5	56.6	1.4	78.9±10.2
Ground, dried, lean meat	61.3	21.5	50	12,028	71.6	1,915	2,346	2,075	38	.31	32.6	29.2±2.5	55.3	5.7	65.7±3.6
All-beef scrap (no. 1)	59.0	21.4	50	9,851	58.6	1,934	2,389	1,994	38	.32	28.8	25.2±2.5	54.5	1.1	80.6±1.0
Meat-and-bone meal (55 percent protein)	55.2	20.4	50	9,461	56.3	1,920	2,349	2,047	36	.31	27.0	23.8±2.5	55.8	2.5	76.5±5.8
Meat-and-bone meal containing no added blood meal or stick	48.3	19.5	50	9,676	57.6	1,932	2,354	2,081	37	.31	28.1	24.8±2.5	53.6	4.0	70.6±2.5
Blood meal and stick	80.6	24.4	50	10,813	64.4	1,936	2,263	2,118	36	.30	30.2	26.7±2.5	53.7	18.9	34.2±4.6

FIFTH YEAR (1933-34)

All-beef scrap no. 2; cooked 4 hours at maximum temperature of 178° F.	56.2	20.0	38	11,122	87.1	2,085	2,546	2,100	36	0.33	29.2	29.7±2.5	55.5	1.9	76.5±2.8
All-beef scrap no. 2A; cooked 6 hours at maximum temperature of 178° F.	57.3	20.3	38	10,900	85.4	2,031	2,407	1,898	33	.34	25.6	26.7±2.5	53.8	1.9	81.8±2.7
All-beef scrap no. 2B; cooked 16 hours at maximum temperature of 178° F.	56.4	19.7	38	11,193	87.7	2,070	2,483	2,052	35	.35	26.6	27.4±2.5	56.4	1.9	73.5±2.7
All-beef scrap no. 2C; cooked 8 hours at maximum temperature of 200° F.	61.3	21.2	38	10,187	79.8	2,091	2,503	2,111	36	.34	25.8	27.2±2.5	55.1	1.9	76.7±5.6
All-beef scrap no. 2+steam-dried blood meal	61.0	20.7	38	10,643	83.6	1,995	2,421	2,085	35	.33	28.0	28.8±2.5	54.9	.9	79.0±3.2
All-beef scrap no. 2+spray-dried blood meal	61.7	20.1	38	10,228	80.1	2,029	2,455	2,015	36	.36	23.4	25.2±2.5	56.7	1.8	70.9±4.3
All-beef scrap no. 2+steam-dried blood meal+liquid stick	58.6	20.6	16	4,896	91.1	2,033	2,513	1,965	35	.31	31.3	31.5±3.9	56.0	.9	67.6±5.4
All-beef scrap no. 2+spray-dried blood meal+liquid stick	58.9	20.8	16	4,333	80.6	2,005	2,529	2,087	32	.30	26.9	28.3±3.9	57.2	1.2	72.5±6.4
A commercial meat-and-bone meal	49.1	19.7	22	6,502	88.0	2,148	2,442	1,904	36	.36	26.9	27.6±3.3	55.9	3.2	70.9±8.5
Liquid stick	56.2	20.4	22	6,610	89.4	2,042	2,452	2,125	38	.38	26.7	27.4±3.3	52.7	6.4	53.8±6.8

The data in table 3 show that in all cases the average egg production and egg weight per bird for the diets containing the meat-fish-milk supplement were greater than for the diets containing no supplement. Also, in the 1932–33 experiment, when it was possible to make direct comparisons between the diet containing the meat-fish-milk supplement and the diets containing the packing-house byproducts, the latter diets produced more eggs per bird than did the former diet. In average egg weight per bird, on the other hand, during the same year the meat-fish-milk supplement gave somewhat better results than did the packing-house products. However, the difference was not significant in either case, nor did the results show any significant differences among the diets containing the various packing-house byproducts. Furthermore, in 1933–34 the test with the all-beef scrap showed that the period and temperature of cooking and the addition of blood meal and liquid stick had no consistent effect on number or weight of eggs.

The smallest eggs per bird were produced on the diet containing the liquid-stick supplement, and comparatively small eggs were produced by birds receiving blood meal and stick and by those receiving meat-and-bone meal containing no added blood meal or stick. However, the eggs produced on most of the supplements tested were of satisfactory commercial weight. The majority weighed 55 g or more, or approximately $1\frac{11}{12}$ ounces.

Table 3 shows that the birds receiving the meat-fish-milk supplement consumed less feed per egg than the birds receiving no protein supplement. However, in 1932–33, when most of the comparisons between the diet containing meat-fish-milk and those containing the packing-house supplements could be made, the birds on the former diet consumed somewhat more feed per egg than those on the latter diets. In the tests with all-beef scrap during the fifth year, there appeared to be no consistent differences in feed consumption as the result of different periods and temperatures of cooking and the addition of blood meal and liquid stick. The pullets receiving the liquid stick as the protein supplement in the diet required the largest quantity of feed per egg produced.

HATCHABILITY

The methods developed by Hendricks [5] for computing the percentage of fertile eggs that hatch and the standard error of this percentage were used in obtaining the values given in the last column of table 3. The data show that the hatchability of eggs produced on the diets containing the meat-fish-milk supplement was greater than on the diets containing no supplement, but the difference was statistically significant in only one case. In general, however, the data indicate that the former diets are definitely superior to the latter for the production of hatchable eggs. Also, in the cases in which direct comparisons were possible, the diets containing the meat-fish-milk supplement were superior in hatchability to those containing the packing-house byproducts except for the diet containing all-beef scrap no. 1.

When compared among themselves, the diets containing the packing-house byproducts showed wide differences in their effect on

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[5] HENDRICKS, W. A. THE STATISTICAL TREATMENT OF HATCHABILITY DATA. Poultry Sci. 14: 365–3 72 1935.

hatchability. In the experiment conducted in 1932–33, the use of ground, dried, lean meat as a protein supplement led to the production of a few more eggs than did the use of the all-beef scrap, but the hatchability on the diet containing the former was significantly less than that on the diet containing the latter. The use of blood meal and stick also led to the production of a few more eggs than did the use of most of the other supplements, but the hatchability was less than half as good as when the other supplements were used.

In 1933–34, the use of the all-beef scrap no. 2A, which was cooked for 6 hours, resulted in a significantly higher hatchability of the fertile eggs set than did the all-beef scrap no. 2B, which was cooked for 16 hours. In the case of all-beef scrap nos. 2 and 2C, which were cooked for 4 and 8 hours, respectively, the differences in hatchability were not statistically significant. When liquid stick was used as the protein supplement, the hatchability of the eggs was distinctly decreased. Additions of blood meal and stick to the all-beef scrap seemed to have a slightly deleterious effect on hatchability, but the results were not uniformly consistent.

SECOND-WEEK EMBRYONIC MORTALITY

The data on second-week embryonic mortality show some noticeably wide variations. In the case of the diets containing the meat-fish-milk supplements, this mortality was comparatively small, being less than 2.5 percent during 3 of the 4 years in which this supplement was used. On the other hand, in no instance was the second-week embryonic mortality less than 5.7 percent in the case of the unsupplemented diets, and in one instance it was 18.1 percent.

For those diets which contained the packing-house byproducts, the range in second-week embryonic mortality was rather wide, apparently because of the diverse nature of the byproducts. The mixture of blood meal and stick, which was fed in 1932–33, resulted in the greatest second-week embryonic mortality of any of the byproducts used. Liquid stick and the meat-and-bone meal purchased on the open market in 1933–34 apparently caused an increase in second-week mortality, as compared with the other products used the same year. Another product which apparently caused an increase in the number of embryo deaths during the second week of incubation was the ground, dried, lean meat used in 1932–33. All lots of all-beef scrap, regardless of the length of time they were cooked or the temperature used, were associated with low mortality during the second week of embryonic life.

In several instances the low hatchabilities were attributable in part to other causes than a high second-week embryonic mortality. This is well illustrated in figure 1, in which the daily mortality, expressed as percentage of fertile eggs set, is plotted against the length of time the eggs had been incubated. The distribution of embryonic mortality, when diets containing all-beef scrap were fed, was approximately normal, that is, it was very similar to that observed when the birds are well fed and are given access to range. In the case of the diet containing blood meal and stick and the one containing liquid stick, the mortality was excessive throughout, and there was not only an increased second-week mortality but an increased first- and third-week mortality as well. The increase in the number of deaths that

occurred during the third week of incubation in the eggs produced with liquid stick as a protein supplement was very pronounced.

LIVE WEIGHTS AND HEALTH OF PULLETS

It is apparent from the data in table 3 that a protein supplement in the diet had some influence on live weight. In all cases, the average maximum live weight attained on the diets containing the meat-fish-milk protein supplement was greater than that attained on the diets containing no protein supplement. Apparently, the packing-
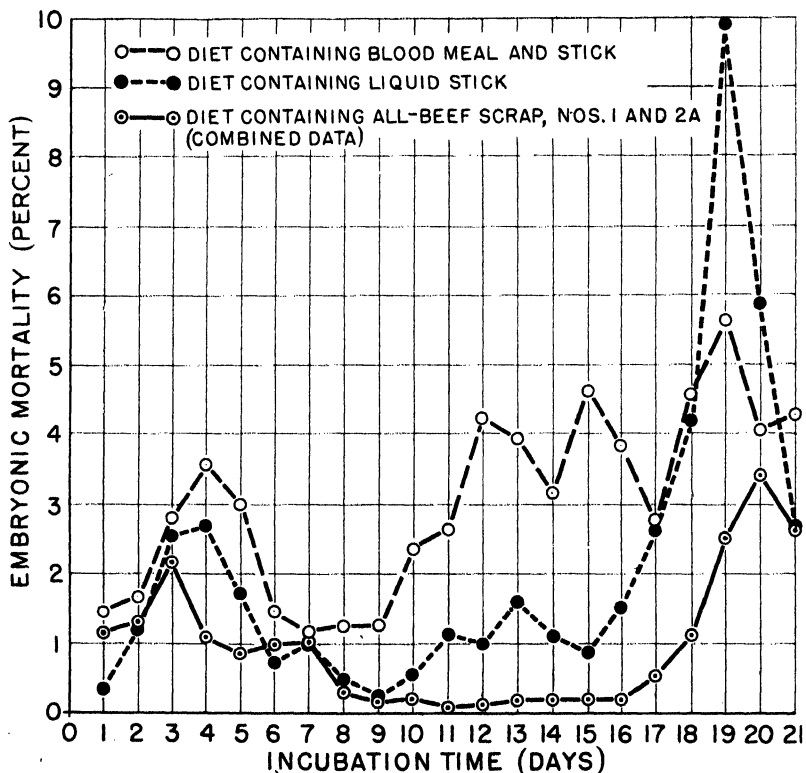
FIGURE 1.—Comparison of mortality of embryos in eggs produced on diets containing blood meal and stick, liquid stick, and all-beef scrap.

house byproducts permitted the birds to reach about the same average maximum live weight as the meat-fish-milk supplement. Although blood meal and stick gave a somewhat lower average maximum live weight than any other diet fed in the fourth year of the experiment, the average final live weight of the birds receiving this supplement was comparatively high.

The average live weight of a flock of birds toward the end of their first laying year is a fair measure of the ability of the diet to maintain live weight in spite of the demands for egg production. In all cases, within the same year, the diets containing the meat-fish-milk supplement were somewhat better than those containing no supplement as well as those containing the packing-house byproducts,

although in 1932–33 the differences were not marked. The results showed no marked differences among the various packing-house byproducts in either average maximum or final live weights. During the last year the final weights in several instances were slightly lower than the initial weights, but both maximum and final live weights attained on all the packing-house byproducts compared favorably with previous results obtained with the positive control, allowance being made for relative initial weights of the pullets.

During the first year, 1929–30, the mortality on all three diets was appreciably lower than is ordinarily observed during the first 336 days after pullets are placed in laying quarters. In 1930–31, however, the disease known as lymphomatosis was first observed among the flocks of chickens on the experiment farm, and in some of the experimental pens there was a marked increase in mortality. During the next 2 years sickness and mortality rapidly increased. In 1933–34 the disease showed a definite tendency to decrease, but it was still somewhat greater than is ordinarily observed in laying flocks. Affected birds were promptly removed from all experimental pens, and the methods of calculation eliminated any appreciable effects of the disease factor on the significant results of the study.

DISCUSSION

One of the most striking results of these experiments was the observation that although several diets supported relatively good egg production, many of the eggs failed to hatch. This appeared to be especially true in the case of diets which contained (1) ground, dried, lean meat, (2) blood meal and stick, and (3) liquid stick. On the other hand, the experiments clearly showed the possibility of preparing byproducts which not only will support good egg production but which will lead also to the production of eggs that hatch well.

In searching for the reason for this difference among packing-house byproducts, the writers found that the nature of the raw materials used in their preparation was a very important factor. But apparent quality from a human-food standpoint did not necessarily mean a satisfactory supplement for the diet of pullets in a breeding flock. For example, the ground, dried, lean meat was a product of excellent quality and considerable care had been taken in its manufacture, yet, so far as hatchability was concerned, it was decidedly inferior to all-beef scrap no. 1, which was made from materials largely unsuitable for human consumption. The liquid stick and the mixture of blood meal and stick were products of a very different kind from those just mentioned, in that stick contains a large percentage of water-soluble compounds, many of which result from the hydrolysis of protein, and no special care is used in its preparation. These two supplements caused an increased second-week embryonic mortality and definitely decreased hatchability.

In the last experiment of the series, the addition of blood meal, or of mixtures of equal parts of blood meal and liquid stick, to the all-beef scrap had no consistent effect on egg production or hatchability, in spite of the fact that both the liquid stick and a mixture of blood meal and stick, when used as the sole protein supplement, decreased the hatchability quite markedly.

So far as the effect of temperature and time of processing were concerned, the few data obtained indicate that cooking temperatures

not greater than 200° F., maintained for not more than 8 hours, were without material effect on egg production or hatchability. There is some indication that prolonged cooking for 16 hours or more, even at a temperature of 178°, may have an unfavorable effect on hatchability.

According to Ellis, Miller, Titus, and Byerly [6] and unpublished data obtained by them, the basal diets used in this study were moderately rich in vitamins B and G. Hence, there is little likelihood that vitamin G was a limiting factor in any of the diets studied. Furthermore, according to the data presented in table 2, the liquid stick used in 1933–34 was a fairly good source of vitamin G inasmuch as it contained practically as much as the different lots of all-beef scrap used in that experiment. Also, these data indicate that the two kinds of blood meal contained about one-half as much vitamin G as the sample of stick and the different lots of all-beef scrap. Therefore, it is evident that the increase in second-week embryonic mortality observed in feeding the diets containing liquid stick and a mixture of blood meal and stick was not due to a deficiency of vitamin G.

Nestler, Byerly, Ellis, and Titus [7] have presented evidence that the basal feed mixture used in these experiments lacks some factor necessary for good hatchability and that this factor is relatively abundant in dried liver and green grass and present to some extent in dried buttermilk. They concluded that dried whey, which is a good source of vitamin G, is not a good source of this factor. Apparently, this factor was well supplied by the several lots of all-beef scrap which were used and which contained some liver.

The value of liver in nutrition has become more and more appreciated during the last decade. This has created a demand for it that has lead to an increase in its cost, with the result that large quantities which formerly were used in making meat scrap and similar products are now sold as dried liver. It would be a good practice for manufacturers to state the liver content of such of their products as do contain liver.

Barnum [8] concluded that vitamin E may be a limiting factor affecting the hatchability of eggs produced on certain diets and that one manifestation of vitamin-E deficiency in chickens is an increased first-week embryonic mortality. In the present experiments, when liquid stick and a mixture of blood meal and stick were used as the protein supplements, there was a definite increase in first-week embryonic mortality (fig. 1). This suggests that the stick and mixture of blood meal and stick may have caused the destruction of some of the vitamin E originally present in the basal feed mixture.

The high third-week embryonic mortality observed in the case of the diets containing stick and a mixture of blood meal and stick was not observed in the case of any of the other diets. The reason for this rather pronounced effect is not known.

SUMMARY AND CONCLUSIONS

A study was made of the effects of packing-house byproducts in the diet of Single-Comb Rhode Island Red pullets on the production and hatchability of eggs. Data were likewise obtained on the effect

[6] ELLIS, N. R., MILLER, D., TITUS, H. W., and BYERLY, T. C. See footnote 4.
[7] NESTLER, R. B., BYERLY, T. C., ELLIS, N. R., and TITUS, H. W. A NEW FACTOR, NOT VITAMIN G, NECESSARY FOR HATCHABILITY. Poultry Sci. 15: 67–70. 1936.
[8] BARNUM, G. L. THE VITAMIN E CONTENT OF EGGS AS RELATED TO THE DIET OF THE HEN AND TO HATCHABILITY. Jour. Nutrition 9: 621–635. 1935.

of such products on live weight, feed consumption, egg weight, and embryonic mortality.

The packing-house byproducts used in the tests included various kinds of meat meal, meat-and-bone meal, beef scrap, blood meal, and stick. These were tested as individual products and several were used in various combinations. The experiment likewise involved comparisons of the effects of different methods of cooking all-beef scrap.

In general, the egg production of birds on diets containing packing-house byproducts was materially higher than that of birds on the basal diet containing no supplement. Egg production resulting from the feeding of packing-house byproducts was somewhat higher than on diets containing a meat-fish-milk supplement known to be efficient in this respect.

In general, the packing-house byproducts, besides supporting reasonably good egg production, resulted in satisfactory hatchability, ranging in most cases between 70 and 80 percent. Decreased hatchability, when encountered, was rather closely associated with an increased second-week embryonic mortality. In the case of the diets containing a specially prepared all-beef scrap, this second-week embryonic mortality was almost negligible.

Liquid stick and a mixture of blood meal and stick led to an increase of embryonic mortality throughout the incubation period and the increase was especially noticeable during the third week.

In their effect on live weight, the packing-house byproducts were about equal. All the byproducts permitted the birds to reach approximately the same average maximum live weight as the meat-fish-milk supplement and the weights of the birds were reasonably well maintained to the end of the experiment.

In their effect on feed consumption per egg produced, the packing-house byproducts were slightly more efficient than the meat-fish-milk supplement. In effect on egg weight, this supplement produced slightly heavier eggs than the packing-house byproducts tested. Most of the packing-house byproducts enabled the birds to produce eggs of satisfactory weight and size. The birds receiving liquid stick produced the smallest eggs. Those receiving a mixture of blood meal and stick and those receiving meat-and-bone meal containing no added blood meal or stick produced relatively small eggs.

The studies indicate that the materials used in making good meat scrap and similar products are relatively more important than the temperature and the time of processing so long as the temperature does not exceed 200° F. or the time of processing 8 hours. Higher temperatures were not studied.

The experiments indicate the commercial possibility of producing excellent packing-house byproducts from the point of view of good hatchability as well as good production. A formula which was found to produce generally satisfactory results consisted of: Carcasses 20 percent, livers 10, spleen 10, skulls 10, beef rennets 15, tripe trimmings 10, hashed pecks 15, and beef-cutting scrap 10.

In general, the experiments confirm previous evidence regarding the value of protein supplements of animal origin, and direct attention to opportunities for improving such supplements by the development and use of suitable formulas and controlled methods of manufacture.

A SEEDLING WILT OF BLACK LOCUST CAUSED BY PHYTOPHTHORA PARASITICA [1]

By EDMUND B. LAMBERT, *associate pathologist, Division of Mycology and Disease Survey*, and BOWEN S. CRANDALL, *assistant scientific aide, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

Large increases in the number of black locust (*Robinia pseudoacacia* L.) seedlings grown in recent years have brought into prominence diseases of this species hitherto overlooked or considered of minor importance. Such a disease developed in 1933 in a Virginia nursery. Many beds were a total loss although resown several times. Toward the end of the epidemic, field observations by Carl Hartley indicated that the disease was a top wilt of plants whose roots still appeared sound. In view of these observations the studies reported in this paper were begun in the fall of 1933.

SYMPTOMS

Opportunities to observe the symptoms of the disease under various conditions have been afforded by epidemics in the field in 1934 and 1935 and by numerous cases resulting from artificial inoculations in the greenhouse.

The severest damage and the most characteristic symptoms of the disease develop in seedlings 1 to 3 weeks old. In seedlings of this age all of the plants may wilt in patches several feet in diameter or even throughout entire beds. Usually the first visible sign of the disease is a slight drooping or curling of the cotyledons (fig. 1, *B*). It is followed, usually in less than a day, by sudden wilting, collapse, and shriveling of the entire upper portion of the seedling (fig. 1, *C* and *D*). Beds that appear to be in fine condition in the evening may be found with large patches of wilted seedlings when examined the following morning.

A day after the wilt develops to the stage indicated in figure 1, *D*, the seedlings have a somewhat conical appearance due to the withered cotyledons and first foliar leaves clinging to the erect stem. At this stage the base of the stem is still turgid. Typically, there is no evidence of a lesion on the stem at the soil surface or of fungus invasion of the roots. The entire seedling withers within a few hours. If beds are not observed regularly, the extent of the loss is not realized, since young wilted seedlings disintegrate rapidly, often almost entirely disappearing in 4 to 5 days.

A preemergence rotting of seedlings also seemed to be a part of the disease complex both in the greenhouse and in the field; but this is not so easy to diagnose nor so apparent in affected beds as the top wilt.
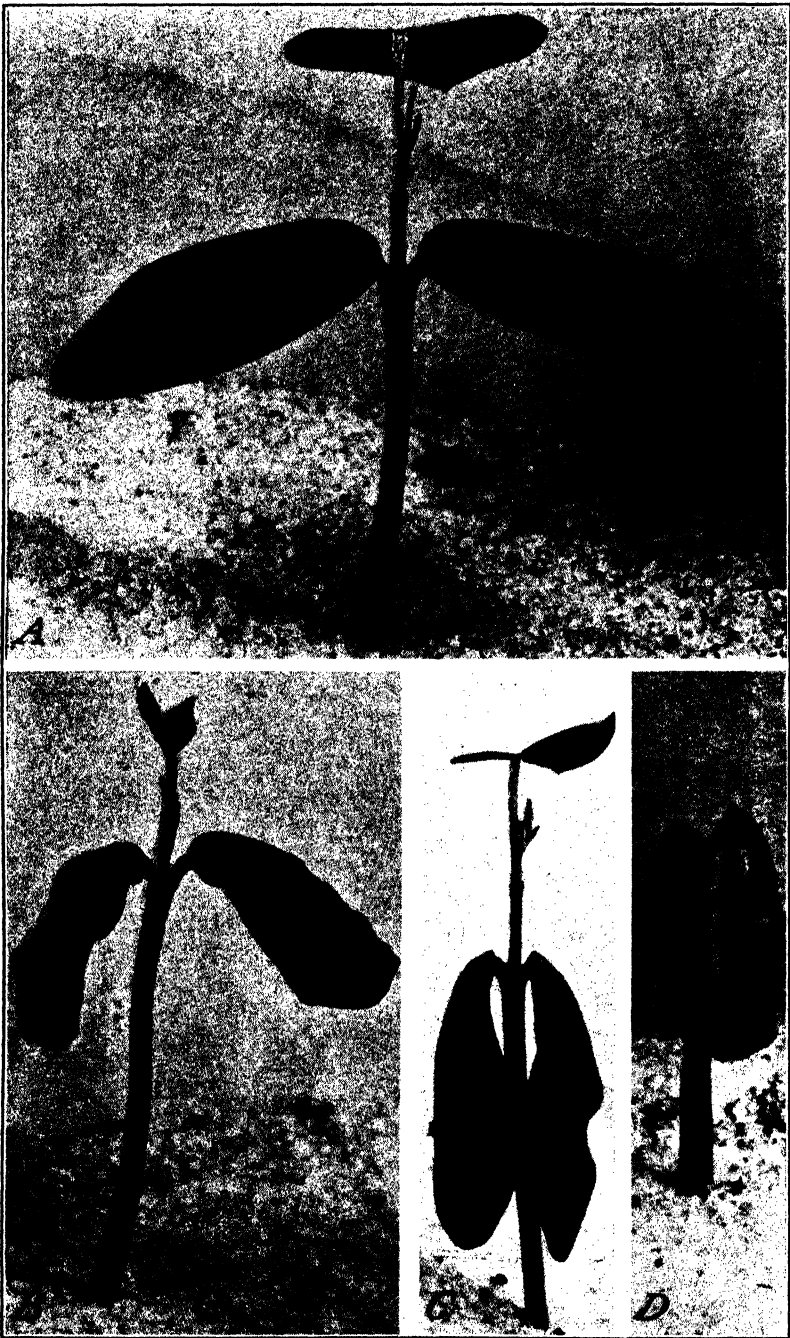
FIGURE 1.—Typical symptoms of wilt disease affecting seedlings 5 days old: *A*, Healthy seedling; *B*, first visible stage of wilt; *C*, cotyledons collapsed; *D*, cotyledons and first foliar leaves wilted but lower stem and roots not yet affected.

After the seedlings are 3 or 4 weeks old, succulent new shoots may wilt rather suddenly here and there in a manner quite similar to the wilting of young seedlings, but the disease is clearly not systemic and is rarely fatal. As the leaves grow older the diseased areas become more localized. Necrotic areas from 5 to 10 mm across may develop on leaves that are otherwise healthy and continue to function. Occasionally leaves will suddenly shrivel as a result of girdling of the petioles. Darkened and sharply defined lesions develop on older stems. These lesions are found at any point on the stem or petiole and frequently remain small and sharply limited without further spread.

Field observations have shown that outbreaks of the disease usually follow a period of cloudy or rainy weather. Typical symptoms usually develop 2 to 4 days after an overnight rain. These observations have been corroborated by studies on the life cycle of the *Phytophthora* causing the disease. During extended periods of cool wet weather, young seedlings may be extensively invaded by fungus hyphae. In such cases, the first symptoms are manifested as soon as the sun begins to dry off the beds, when thousands of seedlings may wither within a few hours.

When diagnosing the disease in beds of young seedlings, care should be taken not to confuse it with the root rot type of damping-off, or with the lesions at the level of the soil surface due to *Rhizoctonia, Pythium*, or possibly *Fusarium*.[3] *Phytophthora* rarely attacks the seedling roots or causes lesions at the soil level, although it is sometimes found mixed with *Rhizoctonia* in such lesions. Drought, heat injury, and the insect injury noted by Snyder and Lamb (5)[4] may also be sources of confusion. The surest diagnostic signs of the disease are the top wilt of young seedlings together with an erect turgid stem base and uninjured root.

ISOLATION AND INOCULATION EXPERIMENTS

Attempts to isolate a pathogen were begun in the greenhouse at Washington, D. C., in the fall of 1933. Soil was shipped in from the diseased beds of the Virginia nursery and sown with black locust seed. Pots containing young seedlings growing on this soil were incubated a few days in a moist chamber. Irregular stands were obtained owing to several causes which at first were rather confusing. However, there were a few wilted seedlings with drooping cotyledons characteristic of the disease as it appeared in the field. A *Phytophthora* was consistently isolated in practically pure culture from the upper parts of these seedlings. As in the field, this wilt affected the cotyledons and juvenile leaves first, then progressed downward into the petiole and upper part of the stem.

Preliminary experiments were made on potted seedlings in order to test the effect of adding the *Phytophthora* culture to uninfected greenhouse soil. It was apparent from these experiments that the *Phytophthora* when growing in the soil could cause a top wilt and, under favorable conditions, a preemergence damping-off.

The presumption was that the top wilt was caused by swarm spores distributed during the watering of the pots. To test this, an

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[3] *Rhizoctonia* was repeatedly isolated from lesions at the soil surface both in the greenhouse and in the field. *Pythium myriotylum* Dresch. (identified by Charles Dreschler) was isolated from diseased seedlings in a North Carolina nursery. *Fusarium* was isolated from the roots of diseased seedlings.
[4] Reference is made by number (italic) to Literature Cited, p. 476.

experiment was made in which 16 seedlings growing in 4 pots were atomized with a water suspension of active swarm spores and a like number were atomized with pure water. The seedlings, which were at the stage of development at which they have cotyledons, a single large juvenile leaf, and small compound leaves (fig. 1, *A*), were then incubated in a moist chamber for 24 hours. Three days later all 16 inoculated seedlings were drooping in a manner typical of the top wilt, and *Phytophthora* was easily isolated from them. The seedlings sprayed with water and incubated at the same time remained healthy.

The evidence obtained from these experiments has been substantiated by numerous subsequent inoculations in the greenhouse, and by isolations made from diseased seedlings in nursery plantings in the summers of 1934 and 1935.

DESCRIPTION OF THE FUNGUS

In the course of the greenhouse studies and subsequent field studies, more than 50 isolates of *Phytophthora* were cultured from black locust seedlings. Although differences in cultural characters were exhibited by different isolates, all of the strains apparently belong to one species. They produce an abundant white aerial mycelium at room temperature on such standard media as oatmeal agar, corn-meal agar, lima bean agar, potato-dextrose agar, steamed rice, and steamed oatmeal. In corn-meal agar the mycelium is nodose and sterile for the first few days, but after 1 or 2 weeks sporangia are produced in moderate numbers. Typical sporangia are prominently papillate and broadly ovate, varying in size around an approximate average of 38μ by 45μ; they are produced in abundance 1 or 2 days after a bit of mycelium, growing on lima bean agar or in locust tissue, is transferred to water. In water they are as a rule nearly spherical; the ratio of length to width is about 1.2 to 1.0. Zoospores are produced in abundance in water cultures subjected to an occasional change of water and kept reasonably free from bacterial contamination. They become active within the sporangia, and swim out at first in a clump and later singly without any evidence of a vesicle. Chlamydospores, usually brown and mostly intercalary, may be found in abundance submerged in cultures on corn-meal agar after 2 weeks' growth at room temperature. They are usually thicker walled than the sporangia and average about 30μ in diameter. No sexual spores were observed, although attempts were made to produce them by crossing all available strains on oatmeal agar and corn-meal agar. The isolates tested have a comparatively high maximum-growth temperature; they continue to grow slowly for at least 4 days at 35° C.

The foregoing attributes indicate that the isolates of *Phytophthora* under consideration are referable to the composite species *P. palmivora* suggested by Leonian (*4*) or to the more limited concept expressed by Tucker (*6*) for *P. parasitica* Dast.[5]

PATHOLOGICAL HISTOLOGY

In order to study the progress of the pathogen within the host, seedlings were grown under aseptic conditions in test tubes and in-

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[5] A culture of one of these isolates was submitted to C. M. Tucker, of the Missouri College of Agriculture, for identification. In his opinion, as expressed in correspondence, it is typical of *P. parasitica*.

oculated with pure cultures of *Phytophthora*. Hyphae were readily distinguished in freehand sections which had been fixed with Carnoy's solution and stained with orange G. Forty-eight hours after inoculation, when petioles of young seedlings were sectioned, the hyphae could be seen advancing in the phloem parenchyma just beneath the epidermal layer and between the cambium cells. At first the mycelium is predominantly intercellular, penetrating the cells with haustoria only (fig. 2). After the hyphae have
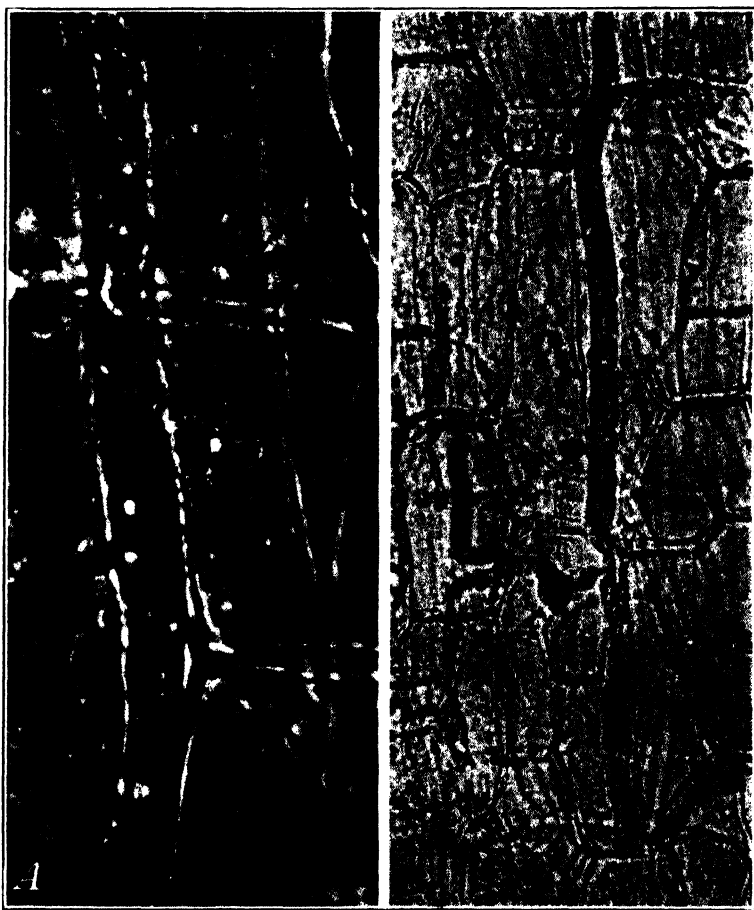
FIGURE 2.—Intercellular growth of hyphae of *Phytophthora parasitica* in phloem parenchyma of 2-week-old *Robinia* seedlings: *A*, Hypha alongside of a cell with a haustorium within the cell, × 1,450; *B*, hyphae following the intercellular spaces, × 350.

progressed a few millimeters, they begin to penetrate the cell walls and shortly thereafter become omnipresent regardless of cell type. Under these conditions, the tissue is soon softened and disorganized.

Experiments in which seedlings of different ages and different degrees of etiolation were sprayed with swarm spores indicate that the pathogen can cause extensive necrosis only in young, succulent host tissue. The sudden dying of numerous shoots frequently observed in the field in month-old plants is due to numerous simul-

taneous infections rather than to a systemic invasion of the plant. The collapse of large compound leaves is usually due to atrophy resulting from the girdling of the petiole by a comparatively small necrotic lesion, rather than to extensive invasion by the pathogen. When compound leaves wilt as a result of hyphal invasion the adja-
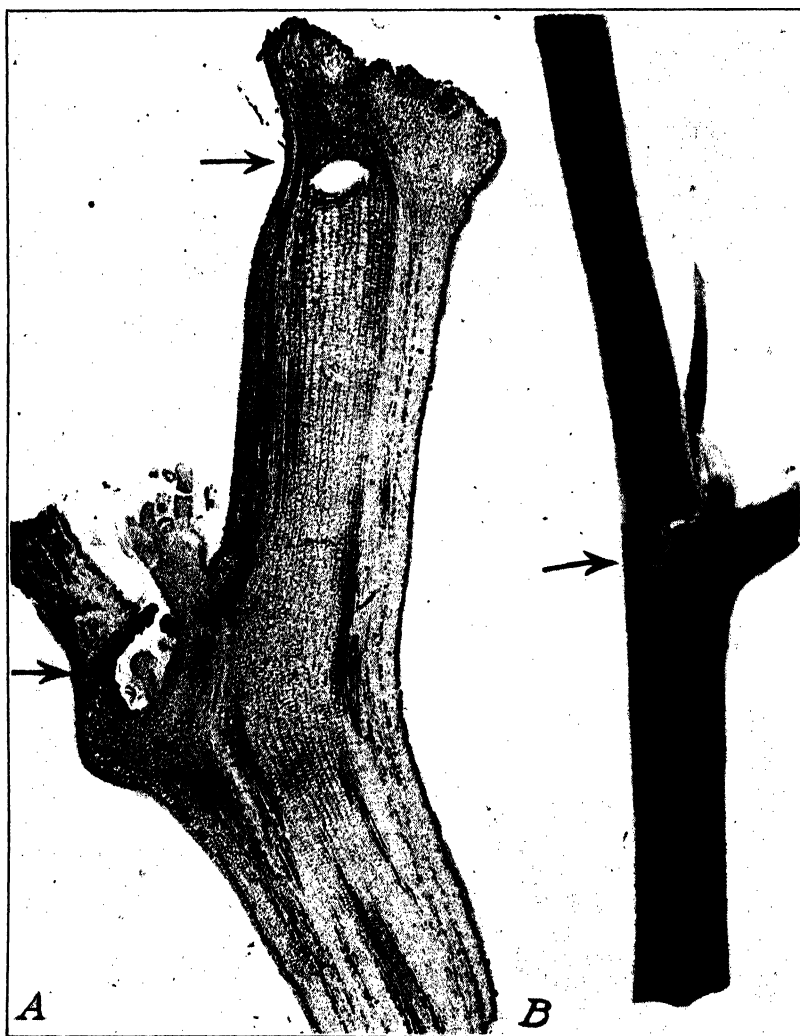
FIGURE 3.—Sections through point of attachment of compound leaf to stem of month-old *Robinia* seedling: *A*, Section stained with safranine and light green, showing result of hyphal invasion from terminal bud in tissue indicated by upper arrow, and the corked-off remains of an infected compound leaf at the point indicated by the lower arrow, × 19; *B*, cutaway view of the base of a normal compound leaf, showing the vascular tissue and cutinized layer tending to isolate the petiole from the bud, × 9. 5.

cent buds remain healthy in a surprisingly large proportion of the cases. Examination of sections made through the point of attachment of the petioles of compound leaves suggests that this is due to the scarcity of parenchymatous connective tissue between the petioles and the adjacent buds (fig. 3).

EPIDEMIOLOGY AND GEOGRAPHIC DISTRIBUTION

Once the fungus is established in a nursery bed, it is evident that prolonged rains during seedling germination favor the spread of the disease. Sporangia and swarm spores are produced 5 or 6 hours after agar cultures are flooded with water. The swarm spores encyst in 5 to 20 minutes after emerging from the sporangia and germinate in 1 to 2 hours. Seedlings have been repeatedly infected with only 4 hours' incubation in a moist chamber after being sprayed with swarm spores. The whole process, therefore, of sporangia production, swarm-spore liberation, migration, encystment, germination, and penetration can easily take place during an overnight rain. Little is known of the resting phases of the life cycle. The fungus presumably lives through unfavorable conditions as chlamydospores, sporangia, dormant mycelium, or oospores in organic matter.

The known distribution of the disease is limited to four nurseries, one in Virginia, two in North Carolina, and one in Alabama. It seems probable, however, that it will soon be found in other places where large plantings of *Robinia* are placed in the natural geographic range of *Phytophthora parasitica*. Tucker (*6*) has assembled information pertinent to this question. He outlines the geographic distribution of this species as typically tropical or subtropical. He further points out that in the United States the species is most generally distributed in the Gulf and South Atlantic States, although it is also found in temperate regions with high humidity, particularly where summer temperatures are high.

Several published references to damping-off of *Robinia* seedlings have come to the attention of the writers. Of these, only the papers of Hartig (*2*), in Germany, and, according to Tucker (*7*), of Collinge (*1*), in England, ascribe the diseases under observation to *Phytophthora* sp. In both these cases, the *Phytophthora* was referred to the *P. cactorum* (L. and C.) Schroet. group rather than to the *P. palmivora-P. parasitica* group. Therefore, as far as the writers have been able to ascertain, there is no published account of a seedling disease of *Robinia* that can be identified with the disease described in this paper.

CONTROL MEASURES

The nature of the disease suggests the possibility of control by soil treatment or by a spray program.

Preliminary tests were made in the greenhouse to determine the effect on the prevalence of the disease of (1) several soil disinfectants, (2) a range of soil acidities, and (3) spraying with sulphur or copper sprays. Of the soil treatments tested, the acidification of the soil with aluminum sulphate appeared to be more promising than any of the disinfectants.

Spray tests were made by first spraying month-old seedlings with a fungicidal spray and later atomizing them at different intervals with active swarm spores of *Phytophthora parasitica*. In these tests protection was obtained with home-made bordeaux mixture (4–6–50), but not with Ansul sulphur spray. Good protection was maintained for at least 2 days after spraying with bordeaux, but infection was not prevented when swarm spores were applied 5 days after spraying, presumably because of the growth of new shoots from under the protective coatings.

In the summer of 1934, three separate series of experiments were made, each in triplicate, to compare the effects of different treatments, which were applied as (1) seed dusts, (2) soil treatments, and (3) sprays. The first series was made in a North Carolina nursery, and the second and third were made in the Virginia nursery where the disease was discovered. Little or no disease developed in the first series, and only a very irregular patchy epidemic developed in the second. In the third experiment, several buckets of soil from part of a bed badly diseased in the previous experiment were spread over the test beds before the treatments were applied. This, combined with suitable weather conditions, produced a uniform epidemic which killed nearly 100 percent of the seedlings in all of the untreated plots. The first two experiments made it possible to eliminate several obviously unsatisfactory treatments, but since these experiments were not very conclusive only the third is discussed in detail.

In the third series the seed treatments consisted of dusting the seed with zinc oxide, copper carbonate, and red copper oxide. The two soil treatments tested were as follows: Bordeaux dust was raked into the surface layer of soil at the rate of one-fourth ounce per square foot of bed space and aluminum sulphate was raked in dry at the rate of one-half ounce per square foot. At the rate applied, the aluminum sulphate increased the acidity of the soil on an average from pH 6.3 to pH 4.6.

The bordeaux spray was prepared by stirring 4 ounces of Orchard Brand mixture into 1 gallon of water. On plot 10 each application of the spray was made at the rate of three-fourths of a gallon to 100 square feet of bed space. Twice as much of the spray was applied each time to plot 12. Four applications of bordeaux spray were made; the first, 2 days after germination was noticed; the next, 2 days later; the next, 4 days later; and the last, 8 days later. The spray was applied at these intervals in order to keep the plants covered during the critical period. The first two applications, at 2-day intervals, kept the cotyledons covered; the application 4 days later covered the single juvenile leaf; and the application 8 days later covered the young compound leaves.

The seed used was furnished by the nursery. It appeared to be excellent seed, but scarification [6] was necessary to obtain good germination and a uniform time of seedling emergence. In field trials only 2 percent of the unscarified seed germinated, while 26.8 percent of the scarified seed germinated.

The plots were laid out in the order shown in table 1, except that all the plots designated by the letter "a" were grouped in one block, the "b" plots in another, and the "c" plots in another. Plots 10, 12, and 14 were not replicated. At the time the experiment was laid out there did not seem to be much hope of obtaining a uniform epidemic over all the plots, so the bordeaux spray trials (nos. 10 and 12), which seemed to be the most promising, and the copper carbonate seed-dusting treatment (no. 14) were crowded onto a section of one of the beds which had suffered 100-percent loss in the previous experiment. This space was too limited to permit replication.

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[6] The seed was scarified by W. M. Hurst, of the Bureau of Agricultural Engineering, U. S. Department of Agriculture, in a barrel seed scarifier similar to that described by Hurst, Humphries, and McKee (*5*). The seed was scarified for 10 minutes with ½- to ¾-inch gravel in the proportion of 5 pounds of gravel to 1 pound of seed.

The notes taken during the progress of the epidemic are summarized in table 1. The extensive wilting observed on September 11 occurred during a hot spell following a cool rainy period from September 7 to 10. (Almost identical conditions accompanied similar losses in the second experiment.) Three treatments were quite evidently effective in decreasing the percentage of wilted seedlings: Bordeaux dust in the soil, aluminum sulphate in the soil, and bordeaux spray. Both of the soil treatments were more effective against the early wave of infection, which caused the wilt recorded September 11, than against later infection. This suggests that the late infection was due to inoculum that drifted in from neighboring plots. If such is the case, the soil treatments would probably be more effective on large plots or if supplemented by a spray program. The favorable effect of the bordeaux spray program, especially the heavier applications, appeared quite striking in the field. The contrast between plots 10 and 12 and their adjacent check plots was noticeable for a distance of several rods.

TABLE 1.—*Effect of various control measures on the incidence of wilt in test plots under severe epidemic conditions*

Control measures	Plot no.	Total germination	Seedling loss recorded on—							Total loss	Average total loss for a, b, and c
			Aug. 29	Aug. 30	Sept. 2	Sept. 11	Sept. 20	Sept. 25	Oct. 8		
Seed dusts:		*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
	1a	592	0.8	3.4	5.2	87.2	3.4	0	0	100	
Check	1b	616	1.3	1.0	2.0	95.7	0	0	0	100	100
	1c	540	.5	1.6	4.5	89.6	3.8	0	0	100	
	2a	464	1.3	2.5	2.7	89.8	3.7	0	0	100	
Zinc oxide	2b	648	.5	.3	0	99.2	0	0	0	100	97.4
	2c	508	1.2	2.0	2.9	74.2	11.8	0	0	92.1	
	3a	576	.8	3.6	2.4	83.3	7.5	0	0	97.6	
Copper carbonate	3b	768	.5	.5	.5	92.0	5.2	0	1.3	100	89.9
	3c	540	.7	3.3	0	3.7	64.4	0	0	72.1	
	4a	368	1.3	2.4	5.4	67.6	20.2	0	0	96.9	
Red copper oxide	4b	440	1.3	0	4.1	8.0	82.0	0	1.1	96.5	96.0
	4c	544	0	2.1	1.1	23.6	67.9	0	0	94.7	
Soil treatments:											
	5a	532	.7	2.8	2.8	77.8	14.1	0	0	98.2	
Check	5b	528	.7	0	3.2	20.8	73.2	0	0	97.9	97.6
	5c	596	.5	14.9	2.1	24.7	54.6	0	0	96.8	
	6a	508	1.0	4.1	3.9	17.3	63.7	0	0	90.0	
Bordeaux dust	6b	588	.8	4.1	1.2	0	59.9	0	0	66.0	64.7
	6c	412	1.7	1.4	1.0	0	24.4	0	9.7	38.2	
	7a	648	.3	1.1	2.0	0	3.1	9.2	3.1	18.8	
Aluminum sulphate	7b	528	.9	1.9	2.3	0	66.4	0	0	71.5	35.2
	7c	516	1.1	1.1	1.5	0	3.9	0	7.8	15.4	
	8a	532	.4	4.7	2.8	79.0	11.3	0	0	98.2	
Check	8b	532	1.1	0	5.1	63.4	28.6	0	0	98.2	97.9
	8c	508	1.2	1.2	.6	82.4	11.8	0	0	97.2	
Sprays:											
Check	9	456	1.8	8.8	4.4	80.6	4.4	0	0	100	....
Light bordeaux	10	468	1.1	3.6	1.5	2.1	60.5	0	0	68.8	....
Check	11	492	2.8	2.4	90.5	0	0	0	2.3	98.0	....
Heavy bordeaux	12	504	.4	0	0	0	0	10.3	0	10.7	....
Seed dust:											
Check	13	254	0	2.8	3.6	93.6	0	0	0	100	....
Copper carbonate	14	262	0	1.7	7.6	71.9	7.6	0	0	88.8	....

It is apparent that the disease was controlled sufficiently for practical purposes either by acidification of the soil with aluminum sulphate or by the heavy application of bordeaux spray. However, these measures would seem to be economically feasible only in exceptional cases, e. g., in beds that are resown following a severe epidemic.

The acidification experiments suggest that severe epidemics of the disease rarely occur on acid soil. Observations made in forest nurseries in the summer of 1935 substantiate this conclusion. In the writers' opinion the disease can be avoided in most cases simply by selecting well-drained sites for the beds, with soil having an acidity of approximately pH 5.

SUMMARY

In the summers of 1933 and 1934 large patches of black locust seedlings (*Robinia pseudoacacia* L.) in a Virginia nursery wilted from a disease that had not previously been reported. In 1934 a few similar cases also were observed in a North Carolina nursery. In 1935 the disease was found in another North Carolina nursery and in an Alabama nursery.

The severest damage and the most characteristic symptoms of the disease develop in seedlings 1 to 3 weeks old. When the seedlings are a month old or more, new growth may suddenly wilt, but the plants as a whole usually recover.

Strains of *Phytophthora*, identified as *P. parasitica* Dast., were repeatedly isolated from wilted seedlings, and the disease was induced among healthy seedlings by spraying them with swarm spores and by infesting the soil with culture material.

Culture studies and inoculation experiments show that the liberation of swarm spores, encystment, germination, and penetration all take place in 4 hours, indicating that overnight rains provide conditions suitable for dissemination of the pathogen.

The disease was controlled in the greenhouse and in field plots by acidifying the soil to a pH value of 4.6 or by means of a spray program with bordeaux mixture. It is suggested that well-drained sites having a soil acidity of approximately pH 5 be selected for sowing black locust.

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MORPHOLOGICAL AND CHEMICAL STUDIES OF THE BLOOD OF CATTLE IN HEALTH AND DURING ANAPLASMOSIS [1]

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INTRODUCTION

The purpose of the present investigation was to obtain precise data on the total red-cell and white-cell counts, the differential white-cell counts, and the chemical constituents of the blood of cattle during anaplasmosis and in health, and thereby if possible to discover some factors in the behavior of the causative agent that would suggest a method of controlling the disease. The morphological readings were taken by the senior author and the chemical readings by the junior author. Each worked independently, the former at Beltsville, Md., and the latter at Washington, D. C. However, since all the blood samples were drawn at Beltsville under the direct supervision of the senior author, and since the same animals were used in the two investigations, the data are presented in a joint paper. The studies are being continued by the Zoological Division.

REVIEW OF LITERATURE

Smith and Kilborne (15) [2] studied the blood of cattle having anaplasmosis, although they believed the disease to be a mild form or a relapse of piroplasmosis characterized by secondary anemia resulting from the destruction of red blood cells. Theiler (17) demonstrated that these investigators were dealing with a specific disease entirely distinct from piroplasmosis, and proposed the name "anaplasmosis." He confirmed their data on the blood picture, and numerous other authors have likewise confirmed them.

Data on the morphology of the white blood cells were reported by De Kock and Quinlan (4). They studied the reticulo-endothelial system of cattle and sheep having anaplasmosis. In both non-splenectomized and splenectomized cases a monocytosis was characteristic of the clinical reaction and. the period of convalescence. Frequently the observed monocytes, some of which were probably reticulo-endothelial cells, contained phagocytized erythrocytes in various stages of digestion. The authors believed that one of the functions of the reticulo-endothelial cells is thus to destroy infected erythrocytes. Confirmatory evidence for this belief was furnished

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by the removal of the spleen of carriers, for after this part of the reticulo-endothelial system was taken out a relapse occurred. The work of De Kock and Quinlan was confirmed by Rees (*11, 12*).

A thoroughgoing statistical study of the white blood cells of cattle in health and during anaplasmosis was conducted by Sergent and his collaborators (*14*). In order to obtain the normal blood picture, they made 67 counts from 37 healthy cattle between the ages of 18 months and 2 years. The mean total white-cell count per cubic millimeter was 10,056. The mean percentages of the various types of cells, as shown by the differential count, were as follows: Lymphocytes, 59.50; monocytes, 7.50; neutrophiles, 22.00; eosinophiles, 10.00; and basophiles, 1.00. Eight cases of anaplasmosis were studied, in seven of which there was a mild leucocytosis, the increase above the average normal figure varying from 1,000 to 10,000 cells. In the differential count during the incubation, clinical, and convalescent periods there was little change from the normal. The average percentages for the clinical period were as follows: Lymphocytes, 62.85; monocytes, 6.80; neutrophiles, 23.70, with no shift to either the right or the left; eosinophiles, 5.70; and basophiles, 0.95. These figures show a slight increase of the lymphocytes and neutrophiles and a decrease of the eosinophiles, but the deviations were not beyond the normal range. In agreement with this work Livotov (*10*) found a leucocytosis during the clinical period, but in disagreement he reported a marked shift to the left of the neutrophiles and also an aneosinophilia. Stephan and his collaborators (*16*) studied 12 clinical cases and found the average percentages to be as follows: Lymphocytes, 47.17; monocytes, 27; neutrophiles, 25.50; eosinophiles, 0.33; and basophiles, 0. Judging from average normal readings, the data of Stephan et al. for anaplasmosis appear to show a relative decrease of lymphocytes, an increase of monocytes, and a marked decrease of eosinophiles; in 9 of their 12 cases there was an aneosinophilia. To summarize the preceding data on the morphological blood picture: There is general agreement that a leucocytosis occurs during anaplasmosis, but on the differential count there is some disagreement. The data of Sergent and his collaborators, for example, show no significant variations from the normal; those of Stephan and his collaborators show a relative lymphopenia, a marked monocytosis, a shift of the neutrophiles to the left, and an eosinopenia.

On the blood chemistry of cattle either in health or during anaplasmosis little work has been published. The concentrations of the constituents vary considerably in the two sexes, as is well shown by the studies of Amadon (*1*) on so-called milk fever. No complete range of data, covering a relatively large number of animals of different breeds and ages on a fixed diet and extending over a period of time sufficient to show seasonal variations, effect of pregnancy, and the like, exists. Moreover, with the use of various methods, different results have been obtained by different workers, and comparison of results is difficult. According to Braga (*3*), the urea of the blood serum rose above normal during the clinical attack of anaplasmosis and fell below normal during convalescence. Ducloux and Cordier (*5, v. 191*), in abstracts of their researches on the blood chemistry of cattle having anaplasmosis, published the following data on the sugar, urea, and bilirubin. During the crisis of the disease the blood sugar rose above the normal level of about 601 mg to 1,000 mg per liter.

During the late clinical period of a severe case the urea rose from the normal level of about 263 mg to 2,315 mg per liter. By both the direct and the indirect Van den Bergh reactions, progressive increase of serum bilirubin was noted during the clinical period and a decrease during convalescence. Ducloux and Cordier used Fehling solution for the quantitative analysis of the sugar after precipitating the proteins with Patein's reagent. For the urea determination they used a hypobromite method and Yvon's ureometer.

EXPERIMENTAL PROCEDURE AND APPARATUS

In the present work, five healthy bulls between the ages of 1½ and 2 years were used. Experimental control was facilitated (1) by keeping the bulls in a barn which was free from ticks and flies, thereby preventing transmission of infection by such means; (2) by feeding uniform rations; and (3) by drawing the blood always at 8 a. m., before the morning feeding. For each 500 pounds of body weight the daily ration was 2½ pounds of equal parts of oats and wheat bran and 12½ pounds of alfalfa hay. However, during the period of acute clinical symptoms the cattle stopped eating entirely. To evaluate uncontrollable factors producing variability in the constituents of the blood, the following procedure was followed: All five bulls were maintained uninfected for 6 weeks during a preliminary period of testing, from May 23 to July 6, 1934, and one of them, bull 124, was held uninfected throughout the 3 months' time required for the joint morphological and chemical testing and for several months beyond this period. The susceptibility of this bull was then established by injecting virulent blood; a fatal case of anaplasmosis resulted. On July 6 four of the bulls, nos. 120, 122, 123, and 125, were injected intravenously with virulent blood.

For the morphological readings the blood was collected from the ear by lancet puncture directly into the diluting pipettes, the same pipettes and other equipment being used for all the readings, which were taken personally by the senior writer. For the differential count of the leucocytes glass slides were used; Schilling's (13) formula for the neutrophiles was followed. For each chemical analysis two samples of blood were drawn from the jugular vein. The first one of 15 cc was drawn into a test tube, where it was allowed to clot. From this sample the phosphorus, serum proteins, and calcium were determined. From the second sample of 20 cc the oxygen and carbon dioxide content, oxygen capacity, hemoglobin, sugar, and urea were determined; the blood was drawn anaerobically and clotting was prevented as follows: The shank of a glass syringe was provided with 4 mg of dry anticoagulant for each cubic centimeter of blood; the anticoagulant consisted of neutral potassium oxalate, 3 parts, and ammonium fluoride, 1 part (for urea determinations potassium fluoride was substituted for ammonium fluoride). The air was exhausted from the syringe by the plunger, which was oiled with liquid petrolatum, and an airtight cap was fitted on the nipple. While the blood was flowing freely from the bleeding needle, the nipple was uncapped and attached and the syringe filled by venous blood pressure. The syringe was then capped, rotated to mix the anticoagulant, and transported to the laboratory in a box especially designed to carry it upright so that the weight of the plunger was always on the blood. The analyses

were begun within 30 to 40 minutes, and except for the calcium analysis, which required an overnight standing period, were completed on the same day that the blood was drawn.

The filtrate for determination of sugar and urea was prepared by Haden's (8) modification of Folin-Wu precipitation, using 8 volumes of $\frac{N}{12}$ sulphuric acid and 1 volume of 10-percent sodium tungstate solution to 1 volume of blood. The filtrate for calcium and phosphorus determinations was prepared by adding 12 volumes of 10-percent trichloracetic acid to 3 volumes of serum. Sugar was determined by the colorimetric method of Benedict (2); phosphorus by that of Fiske and Subbarow (6); serum proteins by Greenberg's method (7), the proteins not being separated; calcium by the manometric method of Van Slyke and Sendroy (19); urea by the method of Leiboff and Kahn (9); carbon dioxide, oxygen content, oxygen capacity, and hemoglobin by the method of Van Slyke and Neill (18). Carbon dioxide in the serum and that in the hemoglobin were calculated from carbon dioxide and oxygen capacity, respectively, in the whole blood.

Because of the laborious procedure involved in determining urea, no determinations were made for the normal period (May 23 to July 2) of any of the bulls. However, bull 124 was normal throughout the entire testing period, and since urea determinations were obtained on this animal after July 2 it was possible by means of these to determine the effect of the disease on the urea.

EXPERIMENTAL DATA

MORPHOLOGICAL CONSTITUENTS

The morphological data (table 1) obtained during the normal and the incubation periods are in agreement with those of previous investigators. Therefore, only the averages for these periods and only the averages for the control bull 124 are reported. The data of table 1 show that the weights of the cattle were adversely affected by anaplasmosis. During the clinical and subclinical periods, despite the fact that the bulls were young, rapidly growing animals, they actually lost weight.

TABLE 1.—*Morphological constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*

Bull no.	Period	Date [1]	Weight	Anaplasmata per 1,000 red cells	Red-cell count per cubic millimeter	White-cell count per cubic millimeter	Differential white-cell count		Neutrophiles				Eosinophiles	Basophiles	Imperfectly stained or smudged
							Lymphocytes	Monocytes	Juveniles	Band forms	Segmented				
		1934	*Pounds*	*Number*	*Millions*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
120	Normal Incubation	May 23–July 6	610	0	7.24	11,943	62.9	6.0	0	2.1	13.9	12.9	1.5	1.1
		July 6–20	618	0	6.12	11,290	61.9	10.7	0	2.7	11.7	10.5	1.3	1.2
	Clinical	July 23		118	3.88	9,800	61.0	22.0	0	5.0	8.5	2.0	.5	1.0
		July 25	593	120	2.62	11,600	68.0	19.5		3.0	4.5	2.5	.5	2.0
		July 27	593	93	1.79	11,600	65.5	10.0	.5	9.5	6.5	5.0	0	3.0
		July 30	594	78	2.01	13,560	47.0	10.0	5.5	21.0	13.5	2.5	0	.5
	Average		593	102	2.57	11,640	60.4	15.4	1.5	9.6	8.2	3.0	.3	1.6
	Subclinical	Aug. 3		5	3.36	10,560	59.0	17.5	1.5	8.0	10.0	2.5	.5	1.0
		Aug. 6	614	0	3.65	12,160	62.0	16.0	1.0	10.0	6.0	4.0	0	1.0
		Aug. 10		0	3.10	11,040	69.5	10.5	0	2.5	8.5	4.5	.5	4.0
		Aug. 17	587	0	3.30	8,960	78.5	12.0	1.0	0	0	7.0	.5	1.0
		Aug. 22		0	3.48	19,320	70.0	12.0	0	10.5	4.5	3.0	0	0
	Average		600	1	3.38	12,408	67.8	13.6	.7	6.2	5.8	4.2	.3	1.4
122	Normal Incubation	May 23–July 6	302	0	6.29	11,700	81.4	5.9	0	1.1	6.9	2.9	.8	1.0
		July 6–20	326	0	5.83	11,340	76.7	9.2	0	1.0	5.5	6.6	.25	.75
	Clinical	July 23		112	3.64	7,400	62.5	22.0	0	8.0	4.5	.5	0	2.5
		July 25	329	225	1.76	11,600	69.5	10.5	5.5	7.5	6.0	0	0	1.0
		July 27		143	1.31	11,760	45.0	24.5	8.0	12.5	9.5	.5	0	0
		July 30		100	.76	35,040	44.0	29.0	4.0	15.0	7.0	1.0	0	0
	Average		329	145	1.87	16,450	55.2	21.5	4.4	10.7	6.7	.5	0	.9
	Subclinical	Aug. 3		4	1.34	23,440	64.5	8.0	2.5	10.0	14.5	0	0	.5
		Aug. 6	280	0	1.62	8,640	61.0	8.0	3.0	15.0	10.0	.5	0	3.0
		Aug. 10		0	2.15	12,960	82.5	5.5	0	5.5	6.0	.5	0	0
		Aug. 17	303	0	3.76	13,080	80.5	15.0	0	0	2.0	2.0	0	.5
		Aug. 22		0	4.50	20,440	64.5	8.5	0	15.0	9.0	3.0	0	0
	Average		291	1	2.67	15,712	70.6	9.0	1.1	9.1	8.3	1.1	0	.8

[1] On July 6 bulls 120, 122, 123, and 125 were injected with virulent blood. Bull 120 reacted to anaplasmosis on July 21; bulls 122 and 125 on July 23, and bull 123 on July 20.

TABLE 1.—*Morphological constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*—Continued

Bull no.	Period	Date	Weight	Anaplasmata per 1,000 red cells	Red-cell count per cubic millimeter	White-cell count per cubic millimeter	Lymphocytes	Monocytes	Differential white-cell count ‖ Neutrophiles ‖ Juveniles	Band forms	Segmented	Eosinophiles	Basophiles	Imperfectly stained or smudged
			Pounds	*Number*	*Millions*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
123	Normal	May 23–July 6	450	0	7.15	8,069	60.6	6.9	0	3.5	24.3	2.9	.9	.9
	Incubation	July 6–20	482	0	5.90	5,760	60.9	9.7	0	5.4	20.1	2.1	.5	1.3
	Clinical	July 23	478	175	2.88	5,920	66.0	20.0	0	5.0	9.0	0	0	0
		July 25			1.90	6,760	68.0	23.0	0	3.0	2.0	2.0	0	2.0
		July 27	457	148	2.52	9,680	57.0	24.5	2.0	6.0	6.0	1.0	.5	3.0
		July 30		220	1.66	8,520	54.0	26.0	1.0	14.0	3.0	0	0	2.0
	Average		467	136	2.24	7,720	61.2	23.4	.8	7.0	5.0	.8	0	1.8
	Subclinical	Aug. 3	465	3	2.58	8,400	57.5	22.0	1.0	14.5	3.5	0	1.5	0
		Aug. 6		0	2.86	8,640	53.0	17.0	0	15.0	12.0	0	0	3.0
		Aug. 10		0	3.40	7,480	56.5	9.0	0	10.0	20.0	.5	0	4.0
		Aug. 17	497	0	3.60	8,880	73.0	15.0	.5	1.0	8.0	1.5	1.0	0
		Aug. 23	494	0	3.10	8,800	63.0	14.5	0	4.5	16.5	.5	.5	.5
	Average		485	.6	3.11	8,440	60.6	15.5	.3	9.0	12.0	.5	.6	1.5
125	Normal	May 23–July 6	568	0	7.62	10,191	60.1	7.1	0	2.5	25.1	3.4	.8	1.0
	Incubation	July 6–20	612	0	6.62	8,030	65.6	12.0	0	2.9	15.6	2.4	.4	1.1
	Clinical	July 23		26	4.18	9,600	59.0	9.5	0	2.5	25.0	3.0	1.0	0
		July 25		35	4.96	11,320	63.0	23.0	0	1.0	4.5	7.0	1.5	0
		July 27	568	54	4.40	11,600	56.0	22.0	.5	5.5	11.0	1.5	0	2.5
		July 30		90	2.44	12,880	46.0	22.0	0	16.0	11.0	1.0	0	4.0
		Aug. 3		25	1.19	16,900	48.0	25.0	.5	16.0	8.0	0	0	1.0
		Aug. 6 [2]	568	10	1.28	14,560	55.0	25.0	3.0	10.0	5.0	0	0	2.0
	Average		568	38	3.07	12,810	54.5	21.1	.9	8.5	10.9	2.1	.4	1.6
124 (control)	Normal	May 23–July 6	596	0	6.44	11,517	66.9	5.6	0	0	17.6	7.5	1.0	1.4
	Normal control of incubation	July 10–20	637	0	6.11	13,050	67.0	9.4	0	1.5	14.1	6.8	.5	.7
	Normal control of clinical	July 23–30	654	0	6.11	7,760	61.9	10.5	0	1.0	18.5	7.7	1.4	2.5
	Normal control of subclinical	Aug. 3–22	696	0	6.50	9,480	56.5	9.2	0	3.7	18.5	10.4	.7	1.0
Averages	Normal		482	0	7.07	10,476	66.2	6.5	0	2.3	15.6	5.6	1.0	1.7
	Incubation		509	0	6.12	9,105	66.3	10.4	0	3.9	13.2	5.4	.6	1.1
	Clinical		489	105	2.44	12,155	57.8	20.4	1.9	8.9	7.7	1.6	.2	1.1
	Subclinical [3]		459	1	3.19	12,187	66.3	12.7	.7	8.1	8.7	1.9	.3	1.2

[2] Bull 125 was killed on this date to obtain material for pathological study; as it appeared that he would not live through the night.

[3] Averages of bulls 120, 122, and 123.

The temperatures deviated from normal during clinical anaplasmosis only, and the graphs for all the affected animals were very much alike; therefore, the temperature chart of bull 122 only is presented (fig. 1). As figure 1 shows, the onset of the clinical period was marked by an abrupt rise in temperature and the termination of this period by an equally abrupt drop. Comparison with table 1 shows a correspondingly abrupt appearance, increase, and marked decrease of anaplasmata. However, a lack of correlation between the severity of attack and the concentration of anaplasmata in the red cells is strikingly shown by the data for bull 125, as compared with those for the other three bulls, for, although the reaction in bull 125 was severe, the counts for anaplasmata were low. On August 6 this animal was killed to obtain material for pathological study since it appeared that he would not live through the night.
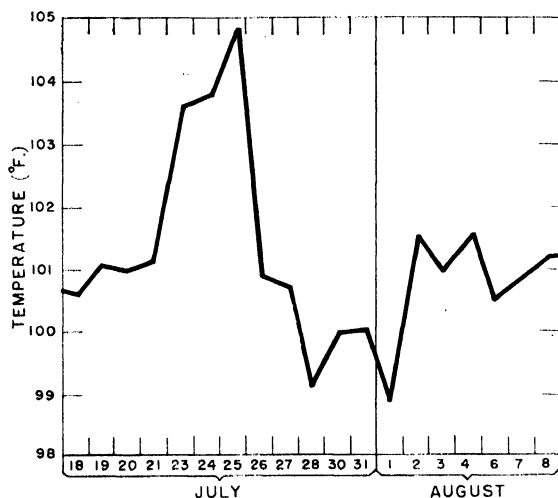
FIGURE 1. Temperature chart of bull 122 from July 18 to August 8, 1934. A positive diagnosis of anaplasmosis was made on July 23 and the clinical period extended from that date to July 30.

In all cases during the clinical period the drop in the red-cell count was very great; in the case of bull 122 it fell below 1 million. It is remarkable that this bull recovered.

In agreement with the data of previous investigators, those of the present paper show a marked leucocytosis during the clinical and the subclinical periods. In showing a marked differential monocytosis, a relative lymphopenia during the clinical period, a shift of the neutrophiles to the left, and a marked eosinopenia, the data of the present writers appear to be in general agreement with those of most of the investigators previously cited, especially with those of Stephan and his coworkers (16), but in disagreement with those of Sergent and his coworkers (14).

CHEMICAL CONSTITUENTS

As pointed out previously, there are no chemical data in the literature with which to compare many of the data of the present paper. For this reason all the chemical data obtained, and the averages as well, are reported in table 2.

TABLE 2.—*Chemical constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*

Bull no.	Period	Date [1]	Quantity of constituent per 100 cc—						Volume of constituent—				Results of Van den Bergh direct test for serum bilirubin
			In whole blood					In serum, calcium	In whole blood, carbon dioxide	In serum, carbon dioxide	In whole blood		
			Sugar	Phosphorus	Serum proteins	Hemoglobin	Urea				Oxygen content	Oxygen capacity	
			Mgs	*Mgs*	*Mgs*	*Gms*	*Mgs*	*Mgs*	*Percent*	*Percent*	*Percent*	*Percent*	
	Normal	*1934* May 23	65.50	7.62	8.66	11.26		12.39	57.58	66.50	6.02	15.59	
		May 29	72.72	8.00	9.81	12.41		11.97	59.26	29.93	9.48	17.14	
		June 4	60.98	8.00	9.45	11.67		14.22	60.57	70.26	5.90	16.14	
		June 12	70.42	7.77	8.00	10.92		11.20	68.82	72.81	8.52	15.14	
		June 26	56.34	6.80	9.63	12.53		10.95	69.83	71.78	8.17	17.31	
		July 2	55.55	6.90	7.65	11.91		11.44	57.26	66.92	8.78	16.46	
			77.52	8.26	9.12	10.85		12.20	59.13	68.00	6.70	15.04	
	Average		65.62	7.54	8.90	11.68		12.05	60.49	63.68	7.37	16.12	
	Incubation	July 10	69.58	6.66	8.66	9.58	23.53	11.89	62.12	70.20	7.17	13.34	Negative.
		July 13	50.00	6.78	8.39	11.82	17.54	11.93	59.53	69.35	8.28	16.34	Do.
		July 17	64.95	6.06	7.88	11.04	12.12	13.56	56.78	65.58	8.13	15.30	Do.
		July 20	59.52	5.26	8.39	9.62	14.39	12.78	60.52	68.51	5.88	13.39	Do.
	Average		61.01	6.19	8.33	10.51	16.89	12.54	59.74	68.41	7.36	14.59	
120	Clinical	July 23	64.10	3.64	7.22	6.96	15.15	12.98	55.33	60.31	3.59	9.70	Delayed, faint.
		July 25	83.68	4.94	9.29	2.79	14.51	11.07	58.01	60.33	1.66	4.24	Delayed, medium.
		July 27	58.57	6.92	7.65	3.66	11.90	10.15	62.29	65.40	.71	5.42	Do.
		July 30	50.00	6.25	6.34	3.79	10.90	11.14	63.83	67.66	1.18	6.37	Delayed, faint.
		Aug. 1	62.11	6.66	7.88	6.14	10.70	10.96	63.67	68.95	1.43	8.73	Do.
	Average		65.09	5.08	7.68	4.67	12.63	11.26	60.63	64.53	1.71	6.89	
	Subclinical	Aug. 3	64.10	6.66	7.54	6.88	12.50	15.67	62.86	68.52	2.61	9.73	Negative.
		Aug. 6	55.87	5.84	7.76	7.53	11.46	17.49	61.31	67.44	2.67	10.59	Do.
		Aug. 10	57.16	6.66	7.88	7.53	12.50	14.24	63.67	69.71	4.27	10.54	Do.
		Aug. 17	62.89	6.40	8.00	6.30	15.38	10.04	63.81	69.19	4.81	8.90	Do.
		Aug. 22	63.30	6.66	9.00	10.92	16.66	11.20	62.82	72.24	8.52	15.14	Do.
	Average		60.66	6.44	8.04	7.83	13.70	13.73	62.89	69.42	4.58	10.98	

	Date											
Normal	May 23	75.40	8.88	8.32	10.68	------	10.90	67.96	78.15	7.70	14.82	
	May 29	81.63	7.27	7.22	10.55	------	11.70	64.27	73.59	7.54	14.78	
	June 4	74.07	9.76	9.45	10.25	------	11.69	59.95	67.83	5.90	14.24	
	June 12	79.37	7.14	10.61	8.98	------	10.96	62.31	69.91	6.62	12.54	
	June 20	62.38	8.25	7.54	11.26	------	10.23	56.51	65.44	8.81	15.60	
	June 26	58.48	6.25	6.50	11.44	------	11.38	62.86	72.92	8.55	15.83	
	July 2	74.07	6.15	6.27	9.41	------	11.00	57.75	65.26	8.02	13.12	
Average		72.20	7.67	7.99	10.37	------	11.12	61.66	70.44	7.59	14.42	
Incubation	July 10	60.97	6.84	7.22	7.86	20.00	11.39	61.09	67.48	6.45	11.04	Negative.
	July 13	57.14	6.72	6.93	10.04	23.53	11.20	57.50	65.55	9.24	13.96	Do.
	July 17	64.31	6.45	6.12	9.79	18.18	12.59	51.88	58.83	8.42	13.62	Do.
	July 20	64.31	5.63	7.03	9.44	19.61	12.49	59.01	66.68	7.76	13.16	
Average		61.68	6.41	6.82	9.28	20.33	11.92	57.37	64.63	7.97	12.94	
Clinical	July 23	62.92	5.71	6.84	6.15	20.83	11.76	61.40	66.31	1.91	8.75	Do.
	July 25	75.19	4.49	6.50	3.31	20.20	9.39	56.23	58.76	.47	4.95	Delayed, strong.
	July 27	44.44	5.13	7.22	1.92	16.20	9.92	46.35	47.74	.24	3.07	Delayed, medium.
	July 30	31.25	6.06	7.27	1.56	25.32	10.65	30.40	31.22	.00	2.59	Diphasic, strong.
	Aug. 1	77.82	7.27	8.66	2.28	30.77	9.98	42.70	44.19	.00	3.56	Prompt, strong.
Average		58.32	5.79	7.10	3.04	22.66	10.34	47.42	49.64	.52	4.58	
Subclinical	Aug. 3	70.67	9.64	6.66	3.00	12.50	11.27	52.42	54.68	.00	4.51	Diphasic, strong.
	Aug. 6	53.62	6.66	7.43	5.01	13.33	16.90	56.85	60.83	1.21	7.22	Delayed, medium.
	Aug. 10	64.93	5.40	8.94	8.94	15.15	15.34	44.28	47.37	2.85	7.12	Negative.
	Aug. 17	75.18	5.00	6.75	6.80	15.38	11.00	65.63	69.57	6.98	9.62	Do.
	Aug. 22	75.47	4.21	7.42	8.98	16.66	12.20	62.31	69.79	6.62	12.54	Do.
Average		67.97	6.18	7.04	6.56	14.60	13.34	56.30	60.45	3.53	8.20	

122

[1] On July 6 bulls 120, 122, 123, and 125 were injected with virulent blood. Bull 120 reacted to anaplasmosis on July 21, bulls 122 and 125 on July 23, and bull 123 on July 20.

TABLE 2.—*Chemical constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*—Continued

Bull no.	Period	Date	Quantity of constituent per 100 cc—						Volume of constituent—				Results of Van den Bergh direct test for serum bilirubin
			In whole blood				Urea	In serum, calcium	In whole blood, carbon dioxide	In serum, carbon dioxide	In whole blood		
			Sugar	Phosphorus	Serum proteins	Hemoglobin					Oxygen content	Oxygen capacity	
			Mgs	*Mgs*	*Mgs*	*Gms*	*Mgs*	*Mgs*	*Percent*	*Percent*	*Percent*	*Percent*	
123	Normal	*1934* May 23	72.70	8.18	7.12	10.86	------	11.15	66.92	77.16	5.05	15.06	
		May 29	82.47	7.62	8.81	11.01	------	10.97	43.23	49.93	8.00	15.26	
		June 4	69.32	7.08	7.22	8.27	------	11.20	61.08	67.49	4.98	11.58	
		June 12	72.46	6.06	6.93	11.06	------	10.23	62.31	72.28	5.44	15.34	
		June 20	54.05	7.92	7.03	11.06	------	10.23	58.04	66.92	5.24	15.34	
		June 26	61.35	6.15	6.66	10.26	------	11.98	53.34	61.02	6.29	14.25	
		July 2	78.12	6.80	6.84	8.69	------	11.97	57.75	64.66	6.87	12.18	
	Average		70.07	7.12	7.23	10.17	------	11.10	57.52	65.64	5.98	14.14	Negative.
	Incubation	July 10	65.75	7.21	7.12	8.01	22.43	11.15	59.55	66.40	5.74	11.91	Negative.
		July 13	56.53	7.02	7.32	10.22	25.00	11.44	50.66	57.90	7.33	14.20	Do.
		July 17	71.43	6.50	7.65	8.51	18.52	12.59	57.07	63.75	5.77	11.91	Do.
		July 20	63.49	5.52	6.30	7.87	21.67	12.78	58.26	64.44	3.54	11.05	Do.
	Average		64.30	6.56	7.15	8.65	21.90	11.99	56.38	63.12	5.59	12.27	
	Clinical	July 23	61.35	5.44	6.75	4.77	19.05	11.93	58.81	62.34	2.05	6.39	Delayed, faint.
		July 25	81.97	6.11	6.50	3.21	22.47	9.87	59.02	61.68	.71	4.94	Delayed, strong.
		July 27	68.26	7.30	10.40	2.60	19.23	10.65	60.53	68.99	.24	3.99	Delayed, medium.
		July 30	61.73	6.66	6.34	4.04	16.95	10.35	57.25	60.11	.47	5.42	Delayed, faint.
		Aug. 1	65.96	6.35	7.76	4.59	15.15	10.96	53.95	57.19	1.19	6.65	Negative.
	Average		67.85	6.37	7.55	3.84	18.57	10.75	57.91	62.06	.93	5.48	
	Subclinical	Aug. 3	66.66	6.15	6.66	5.30	18.18	11.27	54.21	58.00	2.61	7.60	Do.
		Aug. 6	58.82	5.71	6.11	6.27	13.38	13.22	55.28	59.87	2.91	8.90	Do.
		Aug. 10	69.29	6.66	7.42	6.68	16.65	14.20	53.19	57.98	4.75	9.46	Do.
		Aug. 17	62.29	6.15	7.43	6.27	19.05	10.04	61.22	66.16	2.89	8.90	Do.
		Aug. 22	78.74	4.25	7.43	10.57	15.39	11.12	62.31	71.03	5.44	14.67	Do.
	Average		67.14	5.78	7.21	7.02	16.93	11.97	57.24	62.61	3.72	9.91	

Normal	May 23	64.50	7.01	8.66	11.73	---	11.89	69.17	80.58	5.75	16.25	
	May 29	71.43	6.78	7.88	11.69	---	10.90	60.78	70.93	8.51	16.17	
	June 4	57.97	8.08	7.54	11.45	---	12.90	63.63	73.29	5.90	15.85	
	June 12	70.92	5.80	6.12	11.38	---	12.68	63.11	73.21	7.33	15.75	
	June 20	55.10	7.48	7.65	11.02	---	9.99	61.37	70.82	6.43	15.27	
	June 26	51.28	7.27	7.03	12.44	---	10.90	57.01	67.27	4.40	17.18	
	July 2	76.04	7.27	7.76	10.63	---	10.53	63.30	72.67	4.48	14.75	
Average		63.89	7.10	7.52	11.48	---	11.40	62.62	72.68	6.06	15.89	
Incubation	July 10	61.92	7.08	7.76	10.29	20.00	11.39	64.69	73.94	5.97	14.29	Negative.
	July 13	58.48	6.66	7.64	10.04	21.98	10.96	64.12	73.09	8.75	13.96	Do.
	July 17	59.41	6.84	6.93	8.97	15.38	12.10	64.53	72.27	6.71	12.39	Do.
	July 20	47.64	6.50	6.66	9.06	19.32	12.49	60.53	67.98	5.66	12.65	Do.
Average		56.86	6.77	7.25	9.59	19.17	11.73	63.47	71.82	6.77	13.32	
Clinical	July 23	44.74	6.15	7.31	7.66	19.42	12.66	64.44	70.88	4.05	10.90	Negative.
	July 25	74.91	9.64	7.43	6.27	23.53	10.59	70.36	76.20	1.89	8.90	Delayed, faint.
	July 27	57.14	6.66	7.65	4.39	19.30	9.68	66.83	70.84	1.41	6.35	Do.
	July 30	60.24	5.06	6.84	2.81	17.85	10.83	58.01	60.04	.47	3.77	Delayed, medium.
	Aug. 1	62.50	6.02	8.39	1.57	15.63	10.47	57.28	58.61	.00	2.61	Delayed, faint.
	Aug. 3	60.42	4.12	8.66	1.92	15.40	10.04	55.22	56.33	.00	3.08	Do.
125	Aug. 6 [1]	53.48	5.71	8.38	2.32	12.50	14.20	61.53	63.68	.00	3.61	Do.
Average		59.06	6.19	7.81	3.85	17.73	11.21	61.95	65.23	1.12	5.60	

[1] Bull 125 was killed on this date to obtain material for pathological study as it appeared that he would not live through the night.

TABLE 2.—*Chemical constituents of the blood of five bulls in health and of four of them during subsequent anaplasmosis*—Continued

Column groups: "Quantity of constituent per 100 cc" covers *Sugar, Phosphorus, Serum proteins, Hemoglobin, Urea* (In whole blood) and *calcium* (In serum). "Volume of constituent" covers *carbon dioxide* (In whole blood), *carbon dioxide* (In serum), and *Oxygen content* / *Oxygen capacity* (Whole blood).

Bull no.	Period	Date	Sugar (Mgs)	Phosphorus (Mgs)	Serum proteins (Mgs)	Hemoglobin (Gms)	Urea (Mgs)	In serum, calcium (Mgs)	In whole blood, carbon dioxide (Percent)	In serum, carbon dioxide (Percent)	Oxygen content (Percent)	Oxygen capacity (Percent)	Results of Van den Bergh direct test for serum bilirubin
124 (control)	Normal	May 23	66.00	6.73	6.93	10.36		10.40	79.23	91.35	7.19	15.06	
		May 29	62.50	6.15	7.12	11.33		10.22	62.36	72.34	8.27	15.69	
		June 4	61.35	8.00	5.65	10.39		10.23	65.15	74.15	5.22	14.43	
		June 12	67.57	6.72	6.19	10.39		9.73	68.64	78.66	6.15	14.43	
		June 20	55.10	7.48	6.93	11.78	20.83	9.51	62.09	72.21	9.05	16.29	
		June 26	47.62	8.00	7.32	11.48	23.53	11.14	62.10	72.04	6.41	15.89	
		July 2	66.66	7.84	6.50	8.69	13.42	10.77	63.81	71.47	4.24	12.18	
	Average		60.97	7.27	6.66	10.71	19.44	10.29	66.20	76.03	6.65	14.85	
	Normal control of incubation.	July 10	56.66	6.72	7.22	10.11		11.39	58.78	69.01	6.21	14.05	Negative.
		July 13	56.53	6.56	6.27	10.74		10.96	59.02	67.75	8.04	14.90	Do.
		July 17	55.57	7.21	5.84	9.04		12.35	59.92	67.29	6.02	12.62	Do.
		July 20	59.70	6.40	7.32	11.07		12.25	63.05	72.28	6.37	15.34	Do.
	Average		59.61	6.72	6.66	10.24		11.74	60.19	69.08	6.66	14.23	
	Normal control of clinical	July 23	63.09	6.15	6.58	10.22	15.38	13.29	61.62	70.37	6.81	14.20	Negative.
		July 25	71.43	6.61	6.50	11.03	15.87	10.35	59.43	68.64	6.39	15.28	Do.
		July 27	56.66	6.56	7.43	9.79	17.70	(⁴)	60.78	68.99	5.19	13.63	Do.
		July 30	53.19	(⁴)	6.27	9.65	16.07	10.41	61.05	69.27	6.62	13.44	Do.
		Aug. 1	38.82	6.20	8.39	10.33	15.63	10.96	60.35	69.10	7.38	14.48	
	Average		60.64	6.38	7.03	10.21	16.13	11.25	60.65	69.27	6.48	14.21	
	Normal control of subclinical.	Aug. 3	63.69	5.71	7.32	9.51	16.66	11.02	62.61	70.75	5.46	13.25	Negative.
		Aug. 6	54.79	6.15	7.43	8.78	16.66	16.16	61.53	68.91	5.07	12.27	Do.
		Aug. 10	65.57	6.15	7.42	8.80	12.50	13.44	65.40	73.25	7.36	12.30	Do.
		Aug. 17	57.80	6.66	6.93	7.60	16.00	10.19	63.29	69.62	5.33	10.76	Do.
		Aug. 22	66.66	4.22	7.07	10.39	16.66	11.34	68.64	78.94	6.15	14.43	
	Average		61.70	5.78	7.23	9.02	15.70	12.43	64.29	72.29	5.87	12.60	
Average ³	Normal		66.54	7.34	7.66	10.88	19.55	11.19	61.70	69.69	6.73	15.08	
	Incubation		60.96	6.48	7.39	9.51		12.04	59.24	66.99	6.92	13.28	
	Clinical		62.58	6.01	7.53	4.85	17.90	10.89	56.98	66.36	1.07	5.64	
	Subclinical ³		65.26	6.13	7.43	7.13	15.08	13.01	58.81	64.16	3.94	9.70	

³ Averages of bulls 120, 122, and 123.

⁴ Tube was broken; consequently, no analyses could be made.

The data for the normal chemical constituents show a wide range of variation, especially marked in the sugar and gases. During anaplasmosis the deviations from the normal range were slight in some experiments and were marked in other experiments. The oxygen content and capacity of the blood were greatly reduced, complete removal of oxygen occurring in the venous blood of bulls 122 and 125 and almost complete removal in bull 123. The oxygen-carrying capacity, which shows the maximum load that can be taken on in the lungs, is dependent on the hemoglobin. The data show that during the crisis of the disease the available hemoglobin was functioning at its maximum capacity. For example, in bull 122 on July 30, when the hemoglobin was 1.56, or about 15 percent of normal, the oxygen capacity was 2.59, or about 18 percent of normal. Since the carbon dioxide is carried in the serum as well as in the corpuscles this gas was not affected as much as the oxygen. However, in bull 122 during the clinical period there was an appreciable drop in the carbon dioxide content of the serum as well as in whole blood. The range of variation of sugar in the blood during anaplasmosis was in nearly all cases within that found on normal determinations. A comparison of the readings of the affected bulls with those of the normal one shows no significant changes in urea. During the clinical period the Van den Bergh direct reaction was positive in nearly all the readings. In bull 122 on August 1 it was prompt and strong, and in connection with the very low hemoglobin, oxygen capacity, and oxygen content on the same date it shows the extreme gravity of this case of anaplasmosis.

DISCUSSION

For the discrepancies pointed out previously between the morphological data of the present writers and those of Sergent and his collaborators (*14*) the following explanations are suggested: (1) The writers' cases were probably more severe than those of Sergent and his coworkers; (2) different criteria for distinguishing the various types of white cells may have been used (for example, certain cells which the writers classified as monocytes may have been classified by Sergent et al. as large lymphocytes); (3) in certain characteristics bovine anaplasmosis as it occurs in Algeria may be different from that in the United States. However, the morphological data of De Kock and Quinlan (*4*) in Africa, Livotov (*10*) in the Union of Soviet Socialist Republics, and Stephan and his collaborators (*16*) in Brazil appear to be in agreement with those of the present writers. The increase of monocytes and the left shift of the neutrophiles are in accord with current theories of immunity. For the eosinopenia no explanation has thus far been presented.

Discrepancies occur also between the chemical data of the present paper and those of previous investigators. For example, Ducloux and Cordier (*5, v. 192*) noted a ninefold increase of serum urea during the clinical period; in none of the readings by the present writers was there an appreciable increase. Nor do the data of the present writers show an appreciable increase in the blood sugar such as was noted by Ducloux and Cordier. The bilirubin analyses are in agreement. Since the data of the present paper show that the effects of the disease on the blood sugar, phosphorus, serum protein, calcium, and urea were slight, further study should be made of other constituents. For example, the proteins of the whole blood and serum

should be analyzed to determine whether there are significant changes in the albumin and the globulin fractions.

The data of previous investigators and of the present ones all show that clinical anaplasmosis is due primarily to rapid destruction of the red blood cells. This destruction causes a severe drop in hemoglobin and a consequent lowering of the oxygen-carrying capacity of the blood to a point at which it may be insufficient to maintain life. The blood stream soon becomes loaded with infected cells and debris which are converted in part into bilirubin. Most of this is removed in the bile but part of it accumulates in the serum. The reticulo-endothelial system responds by removing infected erythrocytes and debris. Therefore, the outstanding factors in the behavior of the causative agent of anaplasmosis concern its ability to destroy red blood cells, and on this point the present paper does not furnish any information to indicate methods of controlling the disease. Further research is needed to determine how this destruction is accomplished, whether by hemolysins, toxins, or other agents. Within 5 to 10 days after the appearance of symptoms, the destructive agents are counteracted by products elaborated by the etiological agent, by the host, or by both combined. Precise information concerning these counteracting products is also urgently needed.

SUMMARY

This paper records the results of studies made to determine the total red- and white-cell counts, the differential white-cell counts, and the chemical constituents of the blood of cattle during anaplasmosis and health. It was hoped that the results would yield sufficient information regarding the causal agent of the disease to suggest a method of control.

Of five healthy bulls between 1½ and 2 years of age, four were injected intravenously with virulent blood of cattle affected with anaplasmosis, and the fifth was used as a control. Morphological and chemical studies were made of the blood of the five animals for 6 weeks before injections were made and during the course of the disease.

In four affected bulls, during the incubation period of anaplasmosis the readings did not change significantly from the normal.

In general, in the four bulls during clinical anaplasmosis the red-cell count dropped severely; in one case it fell below 1,000,000 red cells per cubic millimeter of blood. The white-cell count increased, the extreme reading being more than 35,000 per cubic millimeter. The differential white-cell count showed a mild lymphopenia, a marked monocytosis, a shift of the neutrophiles to the left, and a marked eosinopenia.

During clinical anaplasmosis the average hemoglobin dropped severely to 3.85 g per 100 cc of whole blood, the oxygen content to 1.07 volumes percent, and the oxygen capacity to 5.64 volumes percent. In one case during the clinical period there was an appreciable drop in the carbon dioxide content of serum and of whole blood. There were no significant changes from the normal in the sugar, phosphorus, serum proteins, calcium, and urea. During this period the serum bilirubin increased until it became detectable by the Van den Bergh direct reaction.

All of the morphological and chemical changes found in the blood of cattle having anaplasmosis were due either indirectly or directly to the rapid destruction of the red blood cells. The nature of the agents causing this destruction was not determined; hence the studies did not indicate a method of controlling the disease.

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A GENETIC FACTOR FOR THE ANNUAL HABIT IN BEETS AND LINKAGE RELATIONSHIP [1]

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INTRODUCTION

Practically all the cultivated varieties of *Beta vulgaris* L., including the sugar beet, show wide genetic variability in respect to the annual and biennial growth habit. Beets that are biennial form vegetative foliar rosettes during the first season of growth. After a period of necessary low-temperature exposure the crown buds of such vegetative beets develop aerial stems bearing spikelike inflorescences. In the annual type this stem development takes place during the first season. These annual types are commonly termed "bolters." Their occurrence is widespread and has been reported from practically all regions of the beet culture in the United States and Europe.

A review of literature indicates that a divergence of opinion exists as to the mode of inheritance of the annual character in beets. As early as 1876, Rimpau (*13*) [3] cited evidence that annual habit is dominant. In a controlled cross between the annual wild beet (*Beta patula* Ait.) and pollen from relatively biennial commercial stock, all the hybrid progeny proved to be annuals. Dudok van Heel (*3*) concluded that bolting factors are recessive. However, this conclusion was not supported by critical breeding data. Vilmorin (*14*) noted marked strain differences in bolting, but from the crosses made he was not able to define the mode of inheritance. Munerati (*10*), in 1931, contributed the first significant advance on the genetics of the bolting character. He crossed several strains which in previous tests had proved to be uniformly annual and biennial, respectively. Some of the biennials used in these crosses were selections from commercial stock, and the annuals were apparently also established from economic sugar-beet varieties. All the F_1 plants were decidedly annual, although showing a somewhat later time of blooming than the annual parent. In summing up Munerati's data which are restricted to reciprocal F_2 progenies, the observed numbers of 3,502 annuals and 1,155 biennials agree closely with the expected 3 : 1 ratio. Munerati concluded that the annual tendency is controlled by a simple Mendelian factor.

The results on the inheritance of the annual character presented in this paper were derived from crosses of one of Munerati's annual strains with several relatively biennial types selected by the writer. Aside from an extension of the simple genetical analysis of the annual habit, the chief contribution relates to a linkage between the factor for annual habit and a factor that chiefly affects hypocotyl and crown

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color. A foliage venation character resembling that of the common plantain (*Plantago major* L.) was also introduced in some of the crosses and gave an opportunity for a study of three contrasting characters.

RELATION OF ANNUAL HABIT TO BREEDING PROBLEMS

The removal of strong bolters in newly selected disease-resistant stocks constitutes a major breeding problem. The need for non-bolting beet varieties suitable for early planting under California conditions is shown by the results of bolting tests conducted by Carsner[4] with two lots of seed grown from the U. S. 1 variety and separated on the basis of time of maturity of the seed. Those plants on which the seed matured early were harvested June 7, 1932. This selection was given the designation V. Selection W, the later maturing lot, was harvested July 8. In a bolting test at Spreckles, Calif., with seed planted January 3, 1933, the final counts on September 6 gave the following bolting percentages: V, 77; W, 23; and the commercial brand Elite Braune, 6. The average weight in pounds per beet at harvest was: V, 1.14; W, 2.23; and Elite Braune, 2.14. In sugar percentage also selection V was the lowest. This evidence shows that in extreme cases bolting results in large losses in yield of sugar.

Results obtained by Esau (*4*) indicated that in the European variety Old Type, grown in central California, the occurrence of a relatively high percentage of bolters may have had very little influence on the final yield. It might be assumed that in this commercial brand selection had removed many of the rapid and hence most undesirable bolting types. The breeding problem now is to combine the nonbolting tendency exemplified by the best European varieties with specific disease resistance to meet conditions where the crop is grown. A demonstration of the heritable basis of the annual habit and the linkage relations to other characters would be helpful in such practical breeding procedures.

MATERIAL

ANNUAL AND BIENNIAL PARENTAL STRAINS

ANNUAL STRAIN

During 1931 two beet strains[5] were tested for bolting. One of these proved to be uniformly annual, even in plantings made as late as June 25 at Salt Lake City, Utah. Figure 1 shows the annual strain in comparison with relatively biennial strains. In subsequent crosses the annual strain proved to be homozygous for both of the simple Mendelian characters—red hypocotyl-crown color and normal pinnate venation—with which the writer has worked extensively.

BIENNIAL STRAINS

The biennials used for crosses are representative of similar types found in economic varieties. A brief history of these biennial strains follows:

Strains 12c21 and 042.—Both of these strains were originally selected by Carsner (*1*) for high resistance to curly top.   In 1931 the writer selected from 12c21 a group of eight stecklings that were relatively nonbolting in habit.   Further tests of the seed increase from these stecklings proved that the selection was a strong biennial type producing no annuals.   This strain was also homozygous for both red hypocotyl color and normal venation.   The original stock of 042 contained an appreciable number of bolting types.   In an early planting of 1930 at Salt Lake City the 042 strain produced no bolters, but other related strains bolted as high as 50 percent.   This indicates the absence of annual types in the 042 strain.   This strain has yellow hypocotyl color and its leaf venation is normal.

Plantain strain.—This strain traces back to a selection from commercial Kleinwanzleben stock made by D. A. Pack, formerly associate agronomist, Division of Sugar Plant Investigations.   It bred true for

FIGURE 1.—Munerati's annual strain (*a*) in bloom, compared with his biennial type (*b*, in the single row to the left of *a*) and with other biennial strains.   Planted at Salt Lake City, Utah, March 24, and photographed June 19, 1931.

a foliage type marked by semiparallel veins.   The foliage venation of the strain under consideration shows prominent lateral veins that are more or less free to the base of the leaf blade, and in this respect it resembles the ribbed condition of the foliage of the common plantain, *Plantago major* (fig. 2).   This selection will be designated in this paper as the plantain strain.   Reselections have been extensively tested for a number of years and have shown no annuals.   This type carried the recessive yellow hypocotyl-crown color.

HYPOCOTYL-CROWN COLOR CHARACTER

The red and yellow hypocotyl-crown color character, which has been mentioned under the description of the annual and biennial parental strains, produces sharply contrasted types.   Keller (*8*) describes the *R* plants as having a rose or pale-red color in seedling hypocotyls.   The bases of stems and petioles also may show this rose or red color.   In large vegetative beets the central bud clearly shows the pigment.   The writer has also been able to detect this color in most types in small buds located in the axils of leaf petioles

and seedstalks. This is a means of classifying plants in the bolting stage, in which the color intensity may be reduced.

The yellow type (r).lacks anthocyan pigment. The hypocotyls are green, with more or less yellow pigment. The intensity of this yellow pigmentation is apparently correlated with exposure to sunlight.

Nuckols (*11*) notes that in young sugar-beet seedlings the color of the red hypocotyl types may vary from a carmine red to light shades of pink. In the seedlings lacking anthocyan pigment the yellow
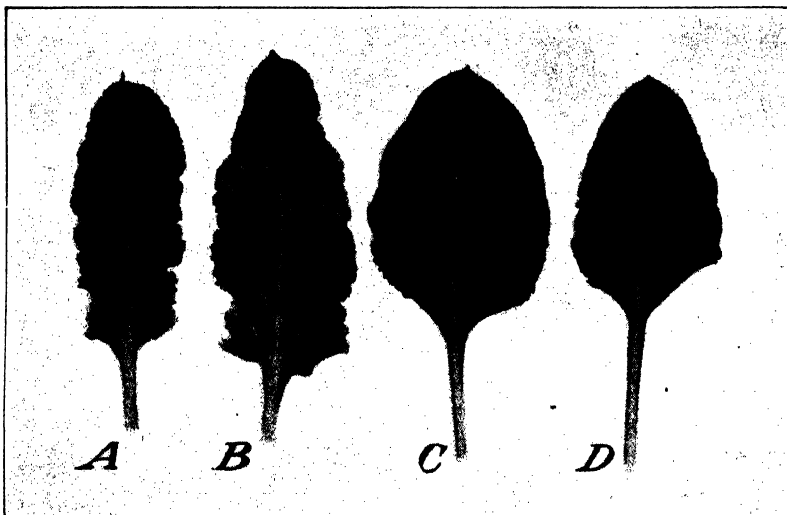
FIGURE 2.—Two leaves (*A, B*) with normal pinnate venation (*Pt*), and two leaves (*C, D*) with semiparallel venation (*pl*) typical of the plantain beet strain. Foliage taken from sib plants in backcross progeny of (*pl × Ptpt*), February 1932.

coloration varies from deep orange to light shades that appear almost white. In a test of 37 varieties Nuckols found that the red and yellow color classes were apparently equal in both root yield and sugar content.

CROSSES

The following crosses and their reciprocals were made:
    Cross 1, annual × 12c21.
    Cross 2, annual × 042.
    Cross 3, annual × plantain strain.

METHODS

CROSSING TECHNIQUE

The types crossed were highly self-sterile. Therefore the methods of distance isolation and bagging proved to be satisfactory for crossing. In the crosses made by the writer, the percentage of contamination with outside strains was very low. The bagging technique was similar to that used in corn breeding. For crosses a simple exchange of bags of the two parents yielded a satisfactory amount of seed. Since self-sterility is not complete under all conditions, the presence of selfed individuals needs to be considered. In crosses such as $rr × RR$, the color character shows the presence of selfs having the

r plant as seed parent and certifies the red plants as crosses. The reciprocal combination does not furnish this information immediately, and a further breeding test is required. In the combination *rr* × *Rr* a large excess of yellow types may be indicative of considerable selfing, and likewise in the *Rr* × *rr* cross an excess of reds may be due to selfs. Such marked deviations, however, occur rarely under conditions of strong self-sterility.

<center>FIELD TESTS</center>

Crosses between the annual type × 12c21, 042, and the plantain strain, respectively, were made in 1932. In 1933, F_1, backcross, and F_2, progenies, as well as parental strains, were tested for annual habit. This material, sown on March 23, was transplanted to the field during the last week of April. The backcross and F_2 plants were either definitely annual or biennial in character, with the exception of four plants that turned vegetative after the initiation of a seed-stalk. This material was classified for bolting on July 5, July 20, and November 11. The last two counts showed close agreement. A field planting of backcross progeny 3492 (F_1 × plantain strain) was made May 31. A count made July 10, only 40 days after planting, showed a good separation into annual and biennial types.

On April 23 and 24, 1934, several progenies and parental strains were sown in small 3-inch pots. Seedlings which had been singled were marked, but in the subsequent field test transplants and undisturbed seedlings showed no difference in the percentage of bolters. Approximately 1,000 of these potted plants were transplanted to the field on May 22 and spaced uniformly from 6 to 8 inches in the row. The material was classified on July 9 and 30. Between these two dates only one plant in all the progenies changed from the vegetative to the flowering state.

On April 26, 1934, several F_2 lots and parental strains were field-sown. The plants were thinned at random to 4 to 6 inches apart in the row. A maximum of bolting was apparently reached on July 22. A previous count on July 2, especially in progenies 3427 and 3432, showed a very large excess of vegetatives. The slow rate of bolting may be partly attributed to a severe infection of curly top to which the plants were exposed. The same F_2 lots were grown under conditions of reduced disease or in the absence of curly top, and in both cases the agreement between the expected and observed annual to biennial ratio was satisfactory.

In both the 1933 and 1934 field tests, check plantings were made of the three parental biennials, 042, 12c21, and plantain strain, respectively, and likewise of the annual type. Out of a total of 320 plants from the biennial strains tested, all remained vegetative. All of the annual parental strain, a total of 241 plants, bolted early. All 84 F_1 plants from crosses 1 and 2 proved to be annual in habit.

<center>GREENHOUSE TESTS</center>

By means of controlled experiments it has been shown by Garner and Allard (6) and Chroboczek (2) that lengthened photoperiods and low-temperature exposure are conditions which, if applied in proper growth stages, increase bolting in beets. Under greenhouse conditions the manipulation of temperature and light serves a useful purpose in genetic studies of growth habit.

The parental strains and the F_1 and F_2 progenies were sown outdoors in flats on August 12, 1933. On September 12, transplants were set in a greenhouse bench and spaced 3 to 4 inches apart in the row. Rows were spaced 8 inches apart. The greenhouse temperatures over the essential period of the test are given in table 1.

Shortly after the plants were set into the bench, additional light was furnished from 100-watt globes spaced 3 to 4 feet apart. From the middle of September to the middle of November these plants received an average of 8 hours of artificial light in addition to the regular daylight.

The separation between annuals and biennials was excellent. In cross 1 (annual × 12c21 biennial) a period of 60 days intervened between the appearance of the last F_2 annual and the beginning of seedstalk formation among the F_2 biennial segregates. In cross 2 (annual × 042 biennial) a period of 40 days separated the completion of bolting by the F_2 annuals and the initiation of seedstalks among the biennial segregates. In cross 3 (annual × biennial plantain strain) an average period of 35 days separated annuals and biennials. In this cross only 2 late and more or less intermediate bolters appeared in a total of 244 F_2 plants tested.

TABLE 1.—*Greenhouse temperatures for September to December 1933*

Month	Maximum	Minimum	Mean	Month	Maximum	Minimum	Mean
	° *F.*	° *F.*	° *F.*		° *F.*	° *F.*	° *F.*
September	90. 2	52. 4	71. 3	November	76. 7	48. 8	62. 8
October	83. 6	43. 7	63. 6	December	73. 7	43. 8	68. 8

In the 1933 greenhouse test, check plantings were made of the three biennial parental strains, 12c21, 042, and the plantain type. A total of 84 plants from the biennial strains remained vegetative during the entire period of the test. In contrast, 65 plants of the annual type all bolted very early.

On April 24, 1934, pot plantings of several backcross and F_2 progenies were made. Until June 21 the greenhouse plants received additional light daily from two 100-watt globes burning from 9 p. m. to 5:30 a. m. On June 21 all vegetative plants were transplanted to the field for further observation. Only 2 plants in a total of 111 classified previously as vegetatives produced seedstalks later in the field.

A small number of F_3 lines were planted October 16, 1934, in a cool greenhouse with additional light. Six red and nine yellow F_2 plants from cross 2, which had been classified in the 1933 greenhouse test as biennials, were backcrossed to yellow and biennial plants. This material was classified for bolting and color January 12, 1935.

EXPERIMENTAL RESULTS

DOMINANCE OF ANNUAL HABIT

Cross 3 (annual × biennial plantain strain) was made in 1932 by means of bagged branches on greenhouse plants. Only a small amount of F_1 seed was obtained. In a field planting of May 5 a

stand of only seven plants resulted, but all were annual in habit, red in color, and normal in venation.   A comparison of the blooming stage reached by the annual parent strain and the F_1 plants showed that while the annual was past full bloom three of the F_1 plants were in full bloom and four in late bud stages.

In the 1933 test, dominance of the annual habit was clearly indicated.   Eighty-four F_1 plants were grown from crosses 1 and 2 (12c21 $\times$ annual and 042 $\times$ annual), respectively.   It was apparent that the F_1 plants were somewhat slower in reaching the full-bloom stage than the annual type planted in the same plot.   Twenty-eight plants of strain 042 and 20 plants of strain 12c21 remained vegetative. Figure 3 shows the relative development reached by the annual

FIGURE 3.— Dominance of annual habit. Rows *a* and *b*, annual and biennial 042 parental strains, respectively; row *c*, F_1 annual plants from cross 042 $\times$ annual.   Transplanted in April and photographed July 5, 1933.

and biennial parental strains and their F_1 progeny from cross 2 (042 $\times$ annual).

The 1933 greenhouse test gave F_1 results in agreement with the field test. Sixty-two F_1 plants from crosses 1 and 2 were definitely annual in habit but slower in developing seedstalks than the annual parental strain.   All greenhouse plantings of the biennial parental strains remained vegetative.

Dominance of the annual habit presents a problem, especially in relation to the comparative rates of seedstalk development of the annual parent, F_1 bolters, and the annual F_2 segregates.   Munerati (*10*) has called attention to the fact that the annual parent develops a seedstalk more rapidly than either F_1 plants or annual F_2 segregates. The writer has gathered some preliminary data on this question also

from material grown in the greenhouse. Spacing of plants in replicated rows was quite uniform. The measurement of seedstalk height was made when the plants of the annual parent strain were nearly all in or near the blooming stage.

The data in table 2 show that the annual strain, with a mean stalk height of 64.54 ± 2.14 cm, is significantly higher in rate of bolting than either F_1 or F_2 annuals. The coefficients of variability of annual and F_1 plants are practically equal. This indicates that with reference to the annual and biennial character the respective parental strains were uniform. However, it is expected that the parental strains could carry factors that would modify heights of inflorescence or time of blooming.

Several hypotheses may be advanced for the apparently incomplete dominance of the annual habit. The most common hypothesis would be that heterozygous Bb plants (B is the factor symbol for annual character and b for vegetative character) are slower in rate of seedstalk development than the homozygotes BB. Some F_2 data in table 2 show that this assumption does not explain all results. With some homozygous types present, one would expect the mean seedstalk height of the F_2 bolters (neglecting the vegetative segregates) to be greater than that for the F_1 annuals. However, this is not the case. In the two F_2 progenies 3430 and 3432, the seedstalk heights are 26.39 ± 1.16 and 22.08 ± 1.78 cm, respectively. The heights of the corresponding F_1 progenies are 33.87 ± 1.66 and 26.00 ± 1.45 cm, in both cases higher than those of the F_2 lots. It is also evident from the 12c21 $\times$ annual cross that F_1 and F_2 progenies are not significantly different in coefficient of variability. The F_2 progeny of the annual $\times$ 042 cross is significantly higher in variability than the F_1 progeny. However, reference to table 2 shows that a preponderance of the higher variability of the annual $\times$ 042 F_2 progeny comes from an increase of plants in the lower seedstalk heights. There is no definite indication from these data that BB plants have increased the average seedstalk heights to any great extent in the F_2 progenies.

A comparison of the rate of bolting of F_1 and F_2 plants needs further consideration. It is possible that F_1 plants possess on an average a greater number of favorable growth factors for height than F_2 plants. Therefore, F_1 plants may not reveal the net effect of the Bb factors on rate of seedstalk development. In comparison, the inbred F_2 plants may show a lower mean seedstalk height, owing to the presence of individuals with a reduced number of favorable growth factors. If a correction could be made for the opposing effects of these growth factors in F_1 and F_2, BB plants might prove to be faster than Bb plants in seedstalk development. Detailed examination of various hybrids in other plants has shown that in many instances dominance is not complete.

Considering the frequency distributions from all three F_2 progenies in table 2, it is clear that out of a total of 143 bolters not over 10 plants approximated the average seedstalk height of the annual parent (64.54 ± 2.14 cm) and that only 1 plant in the F_2 exceeded this. If BB F_2 plants were considerably faster in bolting than Bb annuals, the expectation would be that approximately one-third of the bolters in the F_2 would be near the mean seedstalk height of the annual parent.

TABLE 2.—*Distribution and variability of seedstalk height in crosses between annual and biennial types, greenhouse test, 1933*

Current no.	Strain or cross	Vegetative plants	Plants with seedstalk of indicated height												Total plants with seedstalk	Height of seedstalks		
			6 cm	14 cm	22 cm	30 cm	38 cm	46 cm	54 cm	62 cm	70 cm	78 cm	86 cm	94 cm		Mean [1]	σ	Coefficient of variability
		Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Cm	Cm	
2240	Annual parental type	0				4	1	3	10	8	12	11	1	4	54	64.54±2.14	15.72±1.51	24.4±2.5
2248	F₁ (12c21 × annual)	0		1	2	7	9	2							21	33.87±1.66	7.59±1.17	22.4±3.6
2250	F₁ (042 × annual)	0		3	9	6	3					1			21	26.00±1.45	6.63±1.02	25.5±4.2
3427	F₂ (Plantain × annual)	13	5	12	5	10	7	8	4	2					54	30.52±2.33	17.09±1.64	56 ±6.9
3430	F₂ (annual × 12c21)[2]	9		7	14	19	5	1	3						46	26.39±1.16	7.87±.82	29.8±3.4
3432	F₂ (annual × 042)[2]	20	5	12	15	5	3								43	22.08±1.78	11.70±1.26	53.0±7.1

[1] The mean, standard deviation, and coefficient of variability were calculated from the ungrouped data.
[2] All errors given as standard errors. P. E. = ±0.6745 × standard error.

There is no doubt that as far as initiation of seedstalks is concerned the annual habit is strongly dominant, for F_2 ratios restricted to the strains used in this study have always been 3 annual to 1 biennial. From the data presented it seems that *BB* and *Bb* types do not differ greatly in rate of seedstalk growth. It is possible that other factors for height or maturity exist which depress the rate of seedstalk development in both F_1 and F_2 while not inhibiting initiation of the reproductive phase.

INHERITANCE OF ANNUAL HABIT (CROSS 1)

Table 3 shows that the distribution of annual and biennial plants in F_2 progenies (12c21 × annual) from cross 1 is in very close agreement with the expected 3:1 ratio. It is also apparent that the

FIGURE 4.—Segregation for annual (*A–C*) and biennial (*D–F*) plants in F_2 progeny 3429 (12c21 biennial × annual), October 1933.

greenhouse lots, grown with an increased photoperiod, and the field-grown groups are practically equal in bolting percentage. Figure 4 shows annual and biennial segregates obtained from one of the F_2 progenies grown in the greenhouse.

Under greenhouse conditions the bolters in the F_2 progenies from cross 1 developed rapidly. On September 22, only 14 days after the plants were placed under light and 41 days after the seed was sown, 97 percent of all the bolters were evident. By October 18 only six more plants bore seedstalks. After that date and until December 16, when most of the plants were discarded, no more bolters appeared. The extreme vegetative nature of the F_2 biennial segregates is evident from the following test: After December 16, 10 vegetative segregates of each of the reciprocal F_2 progenies 3429 and 3430 were kept under light for further observation. On January 3, 1934, 77 days from the time the last six bolters appeared, only one plant was definitely starting a seedstalk.

TABLE 3.—*Segregation of annual and biennial habit in F_2 progeny, no. 3429 (12c21 × annual), and the reciprocal, no. 3430* [1]

Current no.	Date and location of test	Total plants	Biennial plants	Annual plants	
		Number	*Number*	*Number*	*Percent*
3429	{1933, greenhouse	98	32	66	67.3
	{1934, field	198	47	151	76.3
3430	{1933, greenhouse	154	36	118	76.6
	{1934, field	176	42	134	76.1
Total observed		626	157	469	74.9
Calculated, 3:1 ratio			156.5	469.5	
Deviation [2]			+.5	−.5	

[1] 3 stecklings each from the reciprocal F_1 lots were grown for F_2 seed in separate field isolations.
[2] $\dfrac{\text{Deviation}}{\text{Probable error}} = 0.07.$

INDEPENDENT ASSORTMENT OF B AND Pl

In cross 3 (plantain strain × annual) the plants differed in leaf venation as well as in growth habit. One of the biennial strains, as previously indicated, was marked by foliage venation similar to that in the common plantain. The inheritance of the plantain venation character is explained on a simple Mendelian basis, with normal pinnate venation dominant. R and Pl are independently inherited. Therefore, B and Pl should likewise show independent inheritance. The results support this expectation.

The backcross data in table 4 are in very close agreement with the calculated $1:1:1:1$ ratio. The F_2 results reported in table 5 are not in satisfactory agreement with the calculated $9:3:3:1$ ratio. It is not a question of linkage, for the poor fit is associated with a large plus deviation of plantain types in the annual class. The deficiency of 13 biennials is not significant. The deviation of biennials is only 2.4 times the probable error. In table 5, according to Fisher's method (5), the observed and calculated frequencies of annuals and biennials are compared separately in the normal and plantain classes. The fit now is satisfactory, χ^2 reducing from 10.766 to 2.851. With two degrees of freedom, P lies between 0.2 and 0.3.

TABLE 4.—*Independent assortment of factors B (annual habit) and Pl (leaf venation) in backcrosses of type bpl × BbPlpl*

Types backcrossed	Current no.	Annual plants		Biennial plants		Total plants
		Normal	Plantain	Normal	Plantain	
		Number	*Number*	*Number*	*Number*	*Number*
$bpl \times BbPlpl$	{2311 2312 2314	31	45	35	35	146
Reciprocal	3492	71	53	68	67	259
Total observed		102	98	103	102	405
Calculated 1:1:1:1 ratio		101.25	101.25	101.25	101.25	
Deviation [1]		+.75	−3.25	+1.75	+.75	

[1] $\chi^2 = 0.146$; P=between 0.98 and 0.99.

TABLE 5.—*Independent assortment of factors B (annual habit) and Pl (leaf venation) in F₂ progenies from matings of the type BbPlpl inter se*

Current no.	Total plants	Annual plants		Biennial plants		Normal [1]		Plantain [1]	
		Normal [2]	Plantain [2]	Normal [2]	Plantain [2]	Annual plants	Biennial plants	Annual plants	Biennial plants
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
2383									
2385	96	50	22	17	7	50	17	22	7
2386									
3427	244	132	64	34	14	132	34	64	14
Total	340	182	86	51	21	182	51	86	21
Calculated		191.25	63.75	63.75	21.25	174.75	58.25	80.25	26.75
Deviation		−9.25	+22.25	−12.75	−.25	+7.25	−7.25	+5.75	−5.75

[1] Theoretical ratio, 3 : 1; $\chi^2 = 2.851$; $P =$ between 0.2 and 0.3.
[2] Theoretical ratio, 9 : 3 : 3 : 1; $\chi^2 = 10.766$; $P =$ between 0.01 and 0.02.

LINKAGE RELATIONS BETWEEN R AND B

BACKCROSS DATA

Cross 2 was made in 1932 in a field isolation between yellow biennial 042 strain × red annual, clone 59. Seed from the two plants was harvested separately. Seventy-five F_1 plants of both combinations were tested and all proved to be annual and red in hypocotyl color. Fifty-four of these F_1 plants came from the white biennial as·seed parent and all showed the presence of the *R* marker, thus certifying the cross in that particular combination.

Cross 3 was originally made in 1932 between two greenhouse plants, yellow biennial plantain venation *rbpl* × red annual pinnate venation *RBPl*. The parental plants were self-sterile. The F_1 plants proved to be annual, and all the F_1 plants backcrossed to the double recessive were found to be heterozygous for *R* and *B*. Figure 5 shows the annual parent that entered into cross 3 and a representative plant from the biennial parental strain. The plantain leaf venation of the biennial strain is evident.

Table 6 gives a summary of data from seven backcross progenies. Seed was obtained by the bagging method. The results clearly indicate linkage in the coupling phase between *R* and *B*. Out of a total of 690 plants observed, 110, or 15.94 percent, were of the recombination classes *Rb* and *rB*. This direct value is practically equal to the 16.1 ± 0.95 percent crossing over as calculated by Owen's (*12*) product-moment method. The cross-over value calculated from the total of cross 2 was 20.0 ± 1.60 percent, the individual values ranging from 18.0 to 25.8 percent. In comparison, the cross-over value of 13.0 ± 1.13 percent from cross 3 was considerably lower.

The total number of annuals and biennials, 342 and 348, respectively, is in very close agreement with the expected 1 : 1 ratio, showing a deviation of only ± 3. This comparison is made in table 7.

The total numbers of observed red and yellow hypocotyl types deviate from the calculated by only ±5. There is therefore no disturbance in the ratios which may be attributed to selfing of either parent in the backcrosses. In the backcross *rb* as female there is no excess of yellow types, and in the *RBrb* female there is no indication of an excess of red-hypocotyl plants. Figure 6 shows annual and

FIGURE 5.—Parental types used for cross 3 differ in three factors: (*A*) Red annual pinnate veins (*RBPl*) × (*B*) yellow biennial plantain veins (*rbpl*).

FIGURE 6.—Annual and vegetative segregates in backcross progeny 2311 (*rb*×*RBrb*). The vegetative plant (*a*) shows the plantain venation character. Photographed July 5, 1933.

vegetative segregates from backcross progeny no. 2311 ($rb \times RBrb$). The vegetative plant happens to be of the plantain venation type, while the three bolters are normal in venation.

TABLE 6.—*Linkage in coupling phase between factors R (hypocotyl color) and B (annual habit) in backcross progenies of the type red annual F_1 (RBrb) × yellow biennial rb and reciprocal and in F_2 progenies of red annual F_1 plants of the type RBrb crossed inter se*

BACKCROSS PROGENIES

Cross no.	Type of mating	Current no.	Red hypocotyl		Yellow hypocotyl		Total plants	Cross-over value [1]
			Annual plants	Biennial plants	Annual plants	Biennial plants		
			Number	*Number*	*Number*	*Number*	*Number*	*Percent*
2	rb×RBrb	3498	58	14	13	58	143	
	do	3499	22	8	9	27	66	
	RBrb×rb	3530	30	3	11	34	78	
	Total		110	25	33	119	287	20.0±1.60
3	RBrb×rb	3492	111	17	14	117	259	
	rb×RBrb	2311 2312 2314	65	12	9	58	144	
	Total		176	29	23	175	403	13.0±1.13
2 and 3	Total		286	54	56	294	690	16.1± .95

F_2 PROGENIES

2	RBrb×RBrb	3432 3535 3536	549 99 206	72 8 17	62 8 6	163 30 34	846 145 263	16.66± .96 11.50±1.92 9.61±1.30
	Total		854	97	76	227	1,254	14.81± .74
3	do	3427 2383 2385 2386	177 64	17 6	19 8	31 18	244 96	17.89±1.86 15.41±2.74
	Total		241	23	27	49	340	17.18±1.54
2 and 3	Total		1,095	120	103	276	1,594	15.29± .67

[1] The cross-over value for the backcrosses calculated according to Owen's (*12*) product-moment correlation. The cross-over value for the F_2 progenies and the probable errors for both backcross and F_2 distributions calculated from Immer's (7) tables.

F_2 DATA

Table 6 gives also a summary of the F_2 linkage data between R and B derived from crosses 2 and 3. Progenies 3432 and 3427 were obtained from field isolations of three and six F_1 stecklings, respectively. Both were grown in plots distant enough from other lots to preclude contamination. The remaining F_2 progenies were obtained from F_1 plants crossed inter se by the ordinary exchange of bags.

The cross-over values, as calculated by Immer's (7) tables, ranged from 9.61 to 17.89 percent. Out of a total of 1,594 F_2 plants, 223 were recombinations, giving a cross-over value of 15.29±0.67 percent. Eleven hundred and ninety-eight plants were annual in habit, and 396 remained vegetative. This is in very close agreement with

the expected 3 annual to 1 biennial ratio. Table 7 shows an F_2 summary of the simple 3 : 1 ratios, including also data from cross 1 (12c21 biennial×annual clone 62). The close agreement is apparent.

TABLE 7.—*Summary of segregation of annual and biennial habit from backcross and F_2 progenies, respectively*

Progenies	Annual plants	Biennial plants	Total plants
Backcrosses:	*Number*	*Number*	*Number*
Total observed	342	348	690
Calculated, 1 : 1 ratio	345	345	690
Deviation	−3	+3	0
F_2 progenies:			
Total observed	1,667	553	2,220
Calculated, 3 : 1 ratio	1,665	555	2,220
Deviation	+2	−2	0

Progeny 3427 (table 6) was also grown in the field in 1934. A total of 635 F_2 plants were classified for color and annual habit. The distribution for bolting is shown in table 8.

The excess of 47 vegetatives, which was evident in both R and r color classes, varies significantly from expectation. The failure to bolt probably lies in the fact that these plants were exposed to an early infection of curly top. The parental strains that were combined in cross 3427 were known to be highly susceptible to this destructive virus disease. Under greenhouse conditions, excluding curly top infection, the same F_2 progeny gave a normal 3 : 1 distribution for growth habit. Under both conditions, the linkage between R and B is apparent. The cross-over value of the field-grown lot under discussion was 19.66 ± 1.21 percent, as compared with 17.89 ± 1.86 percent for the greenhouse material.

F_3 TEST OF VEGETATIVE F_2 PLANTS

In the 1933 greenhouse test, 20 plants were classified as vegetative segregates in an F_2 distribution of cross 2 (annual ×042). Fifteen of these vegetative segregates, six red and nine yellow in hypocotyl color, were backcrossed further with the 042 rb biennial parent strain. An average number of 52 plants per progeny was grown. Out of a total of 777 plants, only 6 bolters were found in 2 progenies. These bolters were of a decidedly slow type as compared with the annual check lot, most of which were in bloom on January 12, 1935. One of the six red F_2 plants proved to be homozygous for color (R). All of the whites bred true for color in F_3. The number of F_2 reds was too small to test the R–B linkage.

TABLE 8.—*Segregation of annual and biennial habit in F_2 progeny 3427 [1] grown in the field in 1934*

Analysis of progeny 3427	Annual plants	Biennial plants	Total plants
	Number	*Number*	*Number*
Total observed	429	206	635
Calculated, 3 : 1 ratio	476	159	635
Deviation	−47	+47	0

[1] See table 6 for greenhouse data.

This F_3 test, with an admittedly small number of progenies, has essentially verified the classification of the F_2 plants in cross 2 as vegetatives. New crosses between annual and biennial types are available now into which a strong self-fertility factor has been introduced. This will greatly reduce the labor of securing an adequate number of F_3 progenies and should give a critical test of the R–B linkage.

DISCUSSION

Interest in bolting has recently been accentuated by earlier commercial planting. West of the Rocky Mountains early sowing is practiced as a means of increasing yields and especially for reducing losses from the curly-top disease. However, in early plantings beets may be exposed to conditions that tend to a marked increase in annual beets. It has been pointed out that bolting is a serious problem in California, where the occurrence of a high percentage of annuals may reduce yields. Bolters are also objectionable to the grower because their presence gives a poor appearance to beet fields. Other tangible objections are encountered in the factory process, since these annual beets, with their woody roots and high fiber or marc content, are difficult to slice. Frequently the presence of a high percentage of bolters results in a pulp of such woody quality that it is unsuitable for stock feed.

The bolting character is so highly sensitive to environmental factors that empirical and superficial selection may often be misleading. Such factors as temperature and light may induce a vegetative condition in beets that inherently possess strong bolting tendencies. For efficiency in commercial breeding operations it is important to recognize genetic factors such as that responsible for the annual habit. Up to the present time the breeding behavior of only a few beet characters has been demonstrated, but, with the great variability existing in sugar beets, genetic investigations should reveal new factors. The presence of a small number of chromosomes in sugar beets, reported by Kuzmina (9) to be nine bivalents, favors the charting of new factors.

The following brief summary of the results presented in this paper shows that the annual habit is dominant over the biennial type and that it is controlled by a single Mendelian factor. A total of 690 backcross plants ($Bb \times b$ and reciprocal) gave 342 annuals and 348 biennials. A total of 2,220 F_2 plants, from matings of the type Bb inter se, showed 1,667 plants in the annual class and 553 biennials. Both the observed backcross and F_2 distributions are in very close agreement with the expected 1 : 1 and 3 : 1 ratios, respectively. The check plantings of annual and biennial parental strains grown under the same conditions as the backcross and F_2 progenies bred true for their respective habits. The results obtained on the inheritance of annual habit verify the conclusion reached by Munerati (10) that the annual tendency is controlled by a simple Mendelian factor.

It has been demonstrated that the F_1 plants and annual segregates from F_2 progenies are slower in rate of development of seedstalks than the annual parental strain. This apparently incomplete dominance of the annual habit, also noted earlier by Munerati (10), does not seem to be highly correlated with a heterozygous Bb constitution.

The hypothesis that modifying factors exert a partial inhibitory effect should be considered in any explanation of the slower rate of seedstalk development of F_1 and F_2 plants.

Keller (8) has established the first linkage group in beets. The cross-over value between the hypocotyl-color factor R and the basic-pigment factor Y was found to be approximately 7.5 percent. The present study has added the annual habit factor B to this first linkage group.

The data for the R and B linkage has been secured from two reciprocal crosses, 042×Muncrati's annual, and plantain strain ×annual. Both of these crosses were of the coupling type $rb \times RB$.

The summarized backcross data from both crosses (table 6) gave a distribution of 286 RB, 54 Rb, 56rB, and 294 rb, and a total of 690 plants. This is obviously in very poor agreement with the expectation on a basis of independent assortment of R and B. The cross-over value from the backcross data proved to be 16.1 ± 0.95 percent. The F_2 results, from a total of 1,594 plants, likewise indicate the R–B linkage, showing a cross-over value of 15.29 ± 0.67. Extreme cross-over values were found to be 9.61 ± 1.30 and 20.0 ± 1.60 percent. The approximate average cross-over value from all data is 15.5 percent.

In cross 3 (plantain strain × annual) the new factor Pl, with which the writer has worked extensively, was introduced. The $Plpl$ factor pair determines dominant pinnate foliage venation versus a semiparallel venation resembling that found in ribbed leaves of the common plantain. R and Pl are inherited independently. Backcross data have indicated that B and Pl are also inherited independently. The agreement with the calculated 1 : 1 : 1 : 1 ratio was very close. F_2 data likewise support the independent relationship of B and Pl.

It is highly probable that the annual factor that the writer has described also operates to produce bolting in many cultivated sugar-beet varieties. The biennial plantain strain used in the present study was derived from commercial stock. The 12c21 and 042 selections with which the writer has worked are relatively biennial in habit and appear to be, so far as the bolting character is concerned, representative of similar types commonly present in sugar-beet stock derived from commercial varieties. The annual strain, as indicated earlier, is probably related to similar annual types which Muncrati (10) has established from commonly cultivated varieties of sugar beets. It is of interest in this connection that, aside from the above-mentioned types, even more divergent forms such as table beets, mangels, and the wild beet (*Beta maritima* L.) intercross readily with sugar beets and appear to be closely related. If the possibility that these related forms have common ancestors is admitted, it follows that homologous genetic factors may exist which produce strikingly parallel variation. In more specific terms, wild beets, table beets, and sugar beets may carry a homologous bolting-factor pair such as Bb. The R and B linkage discussed in this paper offers one means of testing the hypothesis of an isomorphic bolting factor.

The divergent biennial strains that the writer has crossed with the annual type have given essentially identical genetic results as far as inheritance of annual habit and the R linkage is concerned. The apparent correspondence of the bolting character of the biennials used in this study with those commonly present in cultivated sugar-beet

varieties gives ground for the belief that the genetic results of the present investigation may apply directly to the bolting character present in commercial sugar-beet varieties.

SUMMARY

Annual versus biennial habit (Bb) in *Beta vulgaris* is explained on a simple Mendelian basis. The observed numbers of annual and biennial beets in backcross and F_2 distributions were found to agree closely with the expected 1 : 1 and 3 : 1 ratios, respectively. The single-factor basis for the annual character in beets confirms Munerati's results.

Annual habit is dominant. Annuals in both F_1 and F_2 progenies were slower in average seedstalk development than the plants from the annual parental strain. This indicated that dominance, although shifting strongly toward the annual side, may not be complete. The difference between BB and Bb plants in rate of seedstalk development does not appear to be very great. Other factors may exist which partly inhibit seedstalk growth of F_1 and F_2 annuals, while not suppressing the initiation of the reproductive phase.

The B factor assorts independently with Pl, the factor connected with the plantain leaf venation character. A definite linkage of B was noted with the common hypocotyl-crown color factor R. The cross-over value from all data approximates 15.5 percent. This places the factor B in the R–Y linkage group determined by Keller (8).

The biennials used in this study apparently correspond in their bolting character with similar types commonly present in cultivated sugar-beet varieties. It is therefore probable that the genetic results of the present investigation may find direct application to the bolting character present in commercial sugar-beet varieties.

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ZYGOSACCHAROMYCES PINI, A NEW SPECIES OF YEAST ASSOCIATED WITH BARK BEETLES IN PINES [1]

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INTRODUCTION

The present work was undertaken to identify the yeasts that occur in blue-stained wood of trees infested by certain bark beetles. The general association of yeasts and yeastlike organisms with the blue-staining fungi isolated from the wood of beetle-infested trees has been noted by Rumbold (8),[4] and it was with material supplied by her that this study was begun.

Cultures of yeasts were isolated from two species of beetles, *Dendroctonus brevicomis* Lec. from the Western States and *D. frontalis* Zimm. from the Southern States, received at the Forest Products Laboratory during 1932 and 1933. It was found that a heretofore undescribed zygosaccharomycete yeast was generally associated with both species of beetles and the wood of trees infested by them. Some anascosporous, mycelium-forming yeasts not described in this paper, were also frequently present.

During the summer of 1934 a more extensive study of the flora of the beetles of the Southern States confirmed the previous findings for *Dendroctonus frontalis.* In addition it was found that other bark beetles indigenous to the region, *Ips grandicollis* (Eich.), *I. calligraphus* (Germ.), and *I. avulsus* (Eich.), carry the same zygosaccharomycete.

A brief mention of the occurrence of this zygosaccharomycete has been made in a paper by Bramble and Holst (1). Since experimental work is being continued on the function of the yeast in relation to the blue-staining fungi, beetles, and the host, it seemed advisable to present the results of a determinative study at this time. This paper gives a technical description of the organism and a report of its known distribution.

HISTORICAL REVIEW

The first mention of the association of yeasts with blue-staining fungi and bark beetles appears to be that of Grosmann (3), made in 1930. She reported having isolated three nonfermenting types of yeast: (1) A budding yeast having hat-shaped ascospores arising parthenogenetically, (2) a yeast similar to the first type but forming mycelium, and (3) a type forming mycelium but anascosporous. Her first group resembles the organism described in this paper but differs in fermentation and method of spore formation.

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Person (*6*), in 1931, demonstrated the concurrence of yeasts and a blue-staining fungus in beetle-attacked trees, and ascribed to the aroma of the yeast fermentation in the tree the role of attracting fresh insect attacks. No description of the yeast was given, however. Shortly after this, Rumbold (*8*) reported the presence of yeasts and bacteria in cultures of *Ceratostomella pini* isolated from the wood of trees attacked by *Dendroctonus frontalis* and *D. brevicomis*. The association was so intimate that dilution plates made with the ascospores from the perithecia "showed as many bacterial and yeast as fungous colonies." As stated before, many of these yeast cultures were used in the present work.

Recently Leach, Orr, and Christensen (*5*), in a paper on the interrelation of bark beetles and blue stain, called attention to the presence of a characteristic yeast, but gave no details as to the type of yeast. Buchner (*2*) has figured a yeast that forms hat-shaped ascospores, which he found occurring with a cerambycid, *Tetropium castaneum* Paky.

Grosmann's work indicated that the yeasts are not necessary to the life of the beetle as internal symbionts, but may act indirectly by making conditions in the bark more favorable for the developing brood of insects, and, as stated by Person, act as attractants through the fermentation they produce. Preliminary work on the problem, not included in this paper, has also failed to bring to light any evidence of a direct relationship between the yeast and the beetle.

MATERIAL AND METHODS

Cultures obtained from both eastern and western species of bark beetles over a 3-year period were studied to determine whether different species might appear from year to year or whether cultures from different species of beetles varied (table 1). The same species was isolated from year to year from different beetles.· A single-cell isolation made in 1932 from a *Dendroctonus brevicomis* beetle infesting *Pinus ponderosa* Dougl. in Oregon was chosen as the type culture, with which comparisons were made.

TABLE 1.—*Source of cultures of yeast associated with bark beetles*

Blue-stained host	Beetle	Inoculum	Collected in—	
			State	Year
Pinus ponderosa	*Dendroctonus brevicomis*	Beetles and wood	Oregon	1932
Do	do	Beetles	do	1933
Do	do	Wood	California	1932
P. echinata Mill	*D. frontalis*	do	North Carolina	1932
Do	do	Beetles and wood	do	1934
Do	do	do	Alabama	1934
P. strobus L	*D. valens* Lec	Beetles	North Carolina	1934
P. echinata	do	do	Tennessee	1934
P. ponderosa	*Ips oregoni* Eich	do	Idaho	1933
Do	*I. emarginatus* (Lec.)	do	Oregon	1933
P. echinata, P. virginiana Mill.	*I. avulsus*	do	North Carolina	1934
Do	do	do	Tennessee	1934
Do	do	do	Alabama	1934
P. echinata	*I. grandicollis*	do	North Carolina	1934
Do	do	do	Tennessee	1934
Do	do	do	Alabama	1934
Do	*I. calligraphus*	do	North Carolina	1934
Do	do	do	Tennessee	1934
Do	do	do	Alabama	1934

A medium containing 2.5 percent of Trommer's malt extract and 1.5 percent of bacto-agar was used for all the cultural studies. Malt-extract broth of the same concentration was used for the study of growth in liquid medium. Beef-extract-peptone-starch agar was used in determining diastatic action. The fermentation work was carried out in solutions of 10-percent yeast water to which the sugars were added in approximately 2-percent concentration. No special methods were used in studying sporulation, since spore formation appears after a few days on malt-agar slants. Unless otherwise noted, all incubation was at room temperature, 24° to 26° C.

Cultures from wood were obtained according to the method described by Rumbold (7). Beetles were cultured by removing them from freshly exposed galleries in the tree, with either a sterile brush or needle, to malt-agar slants. When fungi were present along with the yeast they could be eliminated by making transfers every 24 hours for several days. If bacteria occurred mixed with the yeasts, a few transfers on malt agar acidified to pH 2.8 to 3.0 would yield a culture of yeast only. Final purification was accomplished by dilution plating, followed by single-cell isolations according to the method of Wright and McCoy (10).

As previously mentioned, all cultural observations were made on malt agar or malt-extract broth. Optimum temperature was determined by inoculating 10-cc amounts of malt-extract broth with equal volumes of a suspension of young cells, counts being made after 24 and 48 hours with a Neubauer blood-counting chamber. Cell size and shape were determined in water mounts. Measurements were made with a filar micrometer.

The action of the yeast on various sugars was tested qualitatively by the Durham tube method, and quantitative determinations of the amount of sugar destroyed were made by the method of Stiles, Peterson, and Fred (9). The Einhorn or Smith tube was also tried initially, but the results were not so reliable as those obtained with the Durham tube.

DESCRIPTION OF ZYGOSACCHAROMYCES PINI, N. SP.

A review of the literature has revealed no named organism with which the one here described can be identified. *Zygosaccharomyces pastori*, first described by Guilliermond (4), bears some resemblance to it in that hat-shaped spores are produced, but the differences in cell size, cultural characters, and sexual mechanism preclude the possibility that the two are identical or even closely related. The name *Zygosaccharomyces pini* is therefore proposed.[5]

DIAGNOSIS

Zygosaccharomyces pini, sp. nov.

Cellulis globosis, ellipsoideis vel ovatis, 2.0–6.4 × 2.5–7μ (in culturis liquidis 2.9–5.5 × 3.6–7μ), gemmantibus; ascosporis 4, post copulationem heterogamicam oriundis, pileiformibus costulatis, sine costula 1.6–2.0 × 2.3–3μ.

In ligno *Pini* spp. cum *Dentroctono* et *Ipse* spp. consociatus, U. S. A. Inducit fermentationem in glucosio, fructosio, et mannosio, nec alliis saccharis vulgaribus.

MORPHOLOGY

In 3-day-old malt-extract agar slants or malt-extract broth, cells round, ellipsoidal, or egg-shaped; cells on slant 2.0μ to 6.4μ by 2.5μ to 7.3μ; in broth, 2.9μ to 5.5μ by 3.6μ to 7.0μ. Asexual reproduction by budding (fig. 1, *A*).
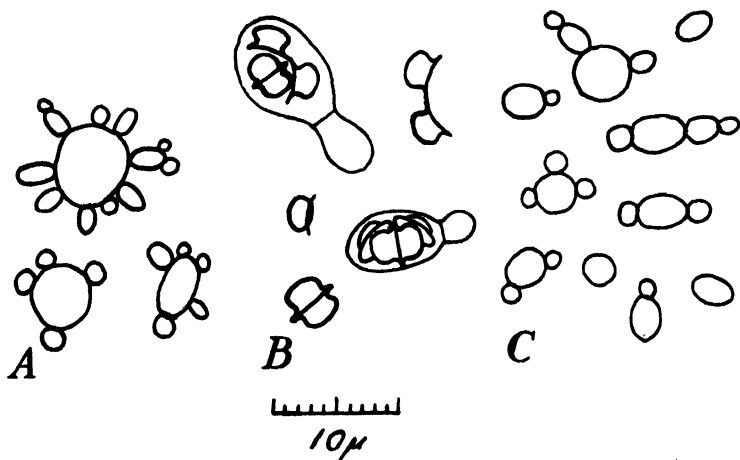
FIGURE 1.— Water mounts of *Zygosaccharomyces pini*, n. sp.: *A*, Cells from a 3-day malt-agar slant culture; *B*, ascospores and asci from older cultures; *C*, multiple budding in an anascosporous strain.

Ascospores formed after heterogamic conjugation. Sporulation occurs readily after several days on malt-extract slants. Asci contain four hat-shaped ascospores measuring, without the brim (fig. 1, *B*), 1.6μ to 2.0μ by 2.3μ to 3.0μ. Spores germinate without fusion, commonly by budding, occasionally by means of germ tube.

CULTURAL CHARACTERS

Slant cultures.—On 7-day-old slant cultures (pl. 1, *A*) growth moderate, filiform, raised, smooth, white, glistening, opaque, butyrous; medium not discolored. In older cultures growth spreading, edge finely lobate, surface may become papillate, color changes from white to fawn (pl. 1, *B*), often with white sectors remaining, perpendicular to line of inoculation.

Broth cultures.—Moderate turbidity, with slight ring present at surface after several days, no pellicle; finely granular sediment.

Giant colony.—Moderate growth, spreading, surface at first smooth, may later become papillate, irregular to lobate edge, color at first white, later fawn-colored, white radial sectors often persisting (pl. 1, *C*).

PHYSIOLOGY

Glucose, fructose, and mannose fermented with production of acid and gas; xylose, arabinose, maltose, galactose, sucrose, lactose, and raffinose not fermented. No hydrolysis of starch. Gelatin not liquefied. No change in litmus milk after 7 weeks.

Maximum temperature for growth on malt agar, 37° to 38° C.; optimum, near 30°. Maximum temperature for sporulation, 34° to 35°; growth and sporulation at 4°.

DISCUSSION

The description given for morphology and cultural characters is that of a recently isolated, freely sporulating culture. When the cultures are carried for a period of from several months to a year or more, anascosporous strains commonly begin to appear, as evidenced by the frequency of white sectors. By repeated transfer from the white sectors it is usually possible to obtain cultures that no longer produce ascospores. Even without such selection certain cultures

PLATE 1
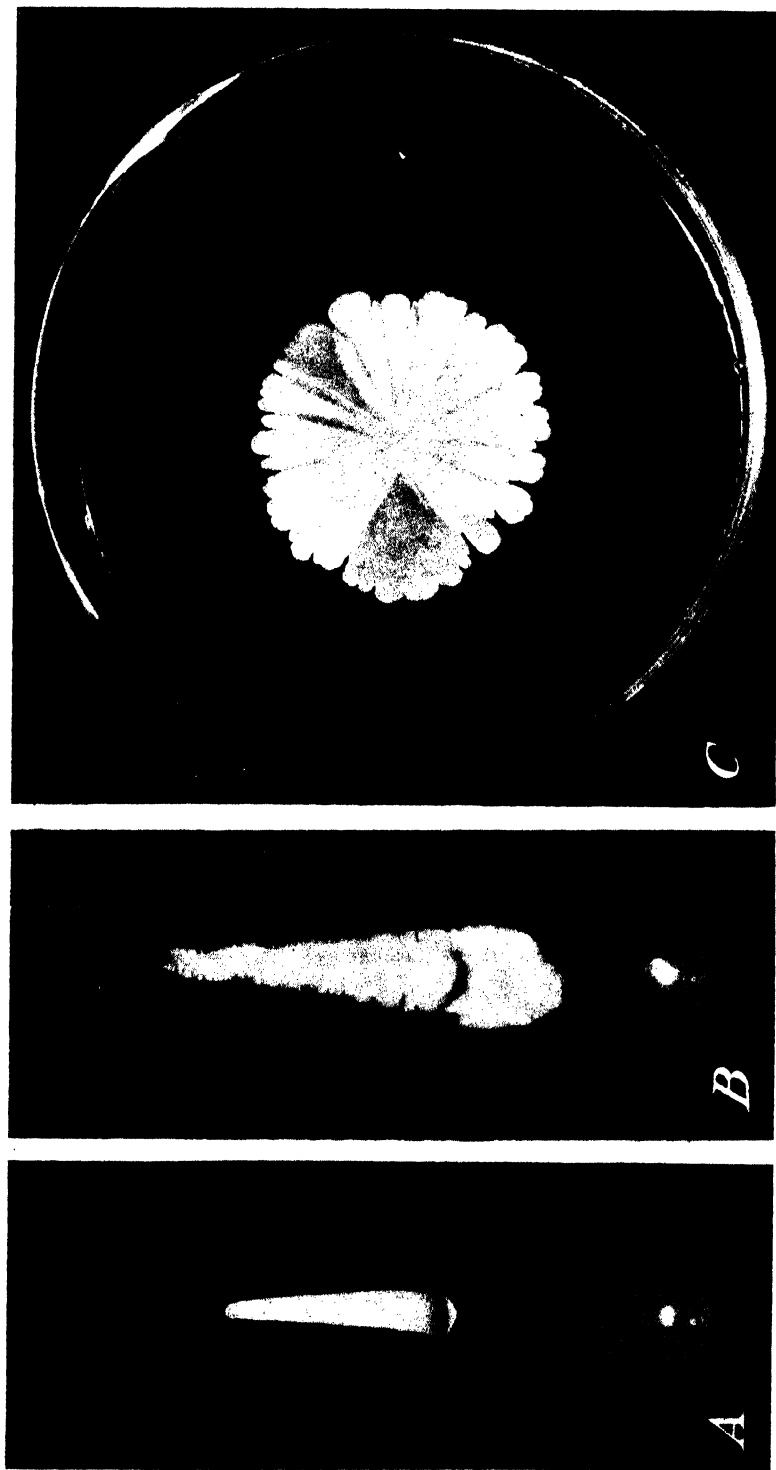
Cultures of *Zygosaccharomyces pini*, n. sp.: *A*, 7-day malt-agar slant culture; *B*, 40-day malt-agar slant culture; *C*, 27-day giant colony on malt agar.

ultimately lose the sexual stage and a distinct change in morphology takes place, by which such cultures may be recognized; that is, large numbers of relatively larger round to slightly oval cells 4μ to 6μ in diameter, exhibiting multiple budding (fig. 1, C), appear. In fact, the appearance of these cells may be taken as a forecast of incipient anascosporogeny. No change in physiology has been found to occur when a culture loses its ascospore-forming property.

There may be a variation, even with freshly isolated cultures, in the rapidity and extent of sporulation. With some cultures ascospores have not been observed until the culture is 2 to 3 weeks old, while in others after only 5 days approximately 95 percent of the cells present were ascospores. Cell size, cultural character, and fermentation properties of these various cultures were found to be in agreement, and no separation into strains appears to be justifiable on the basis of vigor of sporulation alone.

Cultures of *Zygosaccharomyces pini* possess only weak fermentative power. The yeast grows readily on glucose, fructose, and mannose, producing acid and gas in Durham tubes at room temperature, but in many cases gas does not appear until the eighth or ninth day even upon shaking, and from then on the amount increases slowly, reaching a maximum after several weeks. The fact that no acid or gas is produced cannot be taken as evidence that the sugar is not used by the organism. For example, when xylose, arabinose, maltose, galactose, sucrose, lactose, or raffinose yeast-water broths were inoculated with *Z. pini*, neither gas nor acid has produced, yet by quantitative analyses it was found that both xylose and galactose had been utilized, 25 percent of the xylose and 60 percent of the galactose having disappeared after 37 days. The remaining five sugar solutions still contained the original amount of sugars after inoculation and prolonged incubation, although the yeast grew in the solutions. It is of interest to note that the five sugars which the organism can break down, that is, glucose, fructose, mannose, xylose, and galactose, all are known to occur in coniferous woods but not as simple sugars.

SUMMARY AND CONCLUSIONS

A yeast has been found generally associated with the bark beetles *Dendroctonus brevicomis, D. frontalis, D. valens, Ips oregoni, I. emarginatus, I. avulsus, I. grandicollis,* and *I. calligraphus.* A determinative study of the organism showed it to belong to the genus *Zygosaccharomyces,* as ascospore formation is preceded by a sexual process. The formation of hat-shaped ascospores, together with the fact that of the common sugars only glucose, fructose, and mannose are fermented, make it necessary to consider this yeast as a new species. The name *Zygosaccharomyces pini,* n. sp., is therefore proposed, and a description of the cultural, morphological, and physiological characters of the species is given.

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VIABILITY OF SCLEROTIA OF SCLEROTIUM ROLFSII AFTER PASSAGE THROUGH THE DIGESTIVE TRACT OF CATTLE AND SHEEP [1]

By L. D. Leach, *assistant plant pathologist*, and S. W. Mead, *associate animal husbandman, California Agricultural Experiment Station*

INTRODUCTION

In the writers' studies of the agencies involved in spreading *Sclerotium rolfsii* Sacc., the cause of southern sclerotium rot of sugar beets (*Beta vulgaris* L.), circumstantial evidence has indicated that the fungus may be introduced into disease-free areas by cattle and sheep previously pastured on infected beet roots and tops. The present investigations prove that sclerotia can pass through the digestive tract of sheep and cattle without complete loss of viability. The results, however, also indicate that with proper precautions this danger can be greatly reduced or eliminated.

The passage of inert material through the digestive tract of animals has been studied by numerous investigators. Typical curves representing the rate of recovery of ferric oxide and rubber rings from cows were recently presented by Moore and Winter (*6*).[2] That some weed seeds pass through the digestive tract of animals with little or no loss of viability is well known. Several workers have investigated the possibility that plant parasitic nematodes might pass through the digestive tract of farm animals uninjured. The results of Chatin (*1*) and Triffitt (*7*) with *Heterodera schachtii*, of Leukel (*4*) with *Tylenchus tritici*, and of Wilson (*8*) with *T. dipsaci* indicate, however, that these nematodes do not survive passage through animals. Some fungus spores, on the other hand, are known to survive such passage. Ficke and Melchers (*2*) found that from a trace to 2.5 percent of corn smut spores and from a trace to 6.9 percent of sorghum smut spores retained their viability after passage.

EXPERIMENTS WITH SHEEP

PROCEDURE

The four sheep used in the first trial were confined in individual box stalls and equipped with the collection bags (fig. 1) described by Mead and Guilbert (*5*). Their ration consisted of concentrates and alfalfa hay. As soon as the animals were on full feed, 1,000 sclerotia produced from agar cultures were mixed with the concentrates and fed to each sheep at a single feeding. Thereafter only concentrates and hay were fed.

At 12-hour intervals the feces collected from each animal were washed through a series of three screens (10, 20, and 40 meshes to the inch). All sclerotia, except occasional aggregates, passed through the larger screen and were caught in either the 20-mesh or the 40-mesh

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screen. The entire residue from each screen was flushed into a large white porcelain pan with sufficient water to make a depth of 1 inch, and the sclerotia removed with forceps from the extraneous material.

After being surface-sterilized by immersion in $HgCl_2$ 1-1,000 for 45 seconds and washed in sterile water, the sclerotia were plated on potato-dextrose agar to determine their germinability. Early in these trials it was suggested that confinement of the feces in the collection bag (fig. 1) for 12 hours might expose sclerotia to a lethal concentration of ammonia. Analyses [3] revealed, however, that less than 4 p. p. m. of NH_3 was present in the confined feces. Independent investigations (3) have shown that the concentrations mentioned above would not be lethal to sclerotia even with much longer periods of exposure.

TABLE 1.—*Recovery and germination of sclerotia after passage through the digestive tract of sheep,[1] trial 1*

Time after ingestion (hours)	Sclerotia from—							
	Sheep 1		Sheep 2		Sheep 3		Sheep 4	
	Recovered	Germinated	Recovered	Germinated	Recovered	Germinated	Recovered	Germinated
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
12	4	0	1	0.0	2	0.0	12	0.0
24	56	0	72	1.4	55	5.5	60	.0
36	24	0	74	.0	86	.0	99	1.0
48	17	0	27	.0	25	.0	25	.0
60	0	-------	19	.0	11	.0	17	.0
72	0	-------	4	.0	1	.0	7	.0
84	0	-------	2	.0	1	.0	1	.0
96	0	-------	1	.0	0	-------	0	-------
108	0	-------	0	-------	0	-------	0	-------
120	-------	-------	0	-------	0	-------	0	-------
Total	101	0	200	0.5	181	1.7	221	0.5
Controls [2]	-------	90	-------	98.0	-------	98.0	-------	100.0

[1] Each animal consumed 1,000 sclerotia.
[2] Representative samples (50 sclerotia each) of original sclerotia before feeding.

RESULTS OF TRIAL 1

As indicated by table 1 and figure 2, 10 to 22 percent of the 1,000 sclerotia fed to each sheep were recovered. Most of these were passed within the first 48 hours, though one was recovered as late as 96 hours after feeding. In this trial the average recovery was 17.5 percent, of which only 0.71 percent germinated. No viable sclerotia were recovered after the 24- to 36-hour period. Many of the sclerotia recovered after the first 48 hours were broken or fragmented. This observation, together with the fact that only 17.5 percent of the ingested sclerotia were evacuated in identifiable form, led to a slaughter test to secure information concerning the remainder of the sclerotia. Animal 1 was slaughtered at the end of 108 hours, and the contents of the entire digestive tract were washed through screens and examined. As no whole or fractional sclerotia were found, apparently the remaining sclerotia had been so crushed and digested or fragmented that they passed through the 40-mesh screen.

In the second series the conditions of the first trial were altered in two respects. (1) Instead of the small uniform sclerotia formed in pure culture, sclerotia collected from infected sugar beets were used. The latter are often larger and more irregular (fig. 3) than sclerotia from agar cultures. (2) To simulate field-feeding conditions the sclerotia were mixed with chopped sugar beets instead of with dry concentrates as in the previous trial.

Approximately 2,500 field sclerotia were consumed by each of two sheep at a single feeding, and the feces were examined for the presence of sclerotia as in the previous trial. The chopped sugar beets produced an extremely laxative condition in the two animals and during the early part of the experimental period they failed to eat normally. As a result their evacuations were considerably retarded, so that few
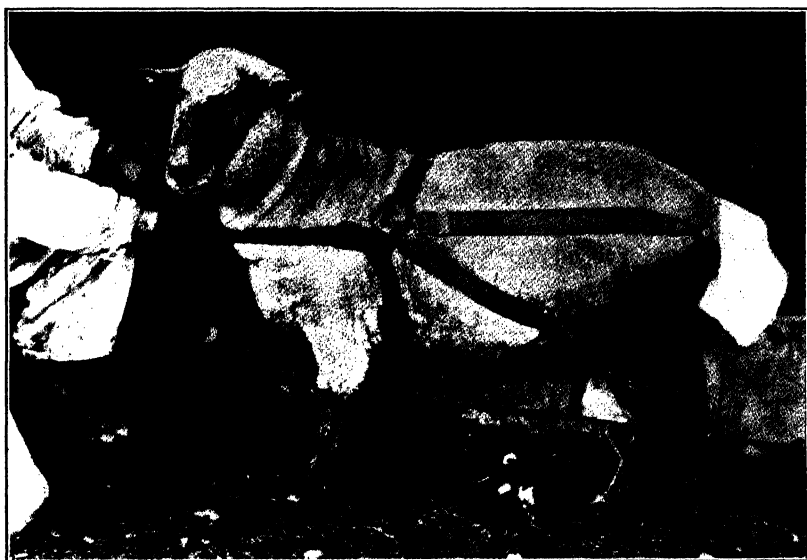
FIGURE 1.—Sheep equipped with apparatus used for collecting feces. Reproduced from California Agricultural Experiment Station Bulletin 409 (*5*).

sclerotia were recovered until the animals were again eating normally. The manner in which the feeding conditions altered the passage of sclerotia may be seen by comparing the results shown in figure 2 for sheep 1, 2, 3, and 4 (trial 1) with those for sheep 5 and 6 (trial 2).

The sclerotia recovered from the two sheep represented 13.3 and 13.5 percent respectively of those consumed, and of the recovered sclerotia 1.6 percent germinated. In this trial some viable sclerotia were evacuated 84 hours after consumption.

As an additional check on the possibility of retention of sclerotia within the digestive tract, animal 6 was slaughtered at the end of 112 hours. When the contents of the digestive tract were examined, as in the previous trial, no sclerotia were found.

The two trials indicate that from 10 to 22 percent of the sclerotia consumed by sheep are evacuated in a whole condition and that a very small percentage of these are still viable. The remainder of the sclerotia, apparently, are broken up into small fragments or digested.

TOXICITY OF RUMEN EXTRACT

Ficke and Melchers (*2*) presented evidence that contact of sorghum smut spores with the contents of the stomach of a horse for 2 hours prevented germination of nearly all the spores. Groups of 100 sclerotia were immersed in the contents of the rumen of animal 6 for 1 to 48 hours, and then tested for germinability. After 1 hour of immersion 100 percent of the sclerotia germinated; after 2 hours, 95 percent; 6 hours, 93 percent; 12 hours, 70 percent; 24 hours, 99 percent; 36 hours, 54 percent; and 48 hours, 85 percent. Similar nontreated sclerotia showed 99 percent germination. These results
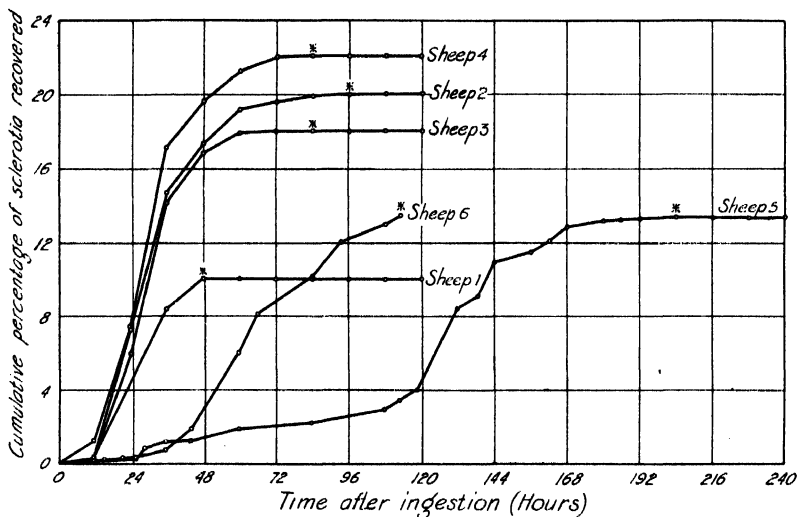
FIGURE 2.—Rate of recovery of sclerotia after passage through sheep. From 10 to 22 percent of the sclerotia fed to the animals were recovered in a whole condition. Animals 1, 2, 3, and 4 (trial 1) were fed dry concentrates and evacuated normally, whereas animals 5 and 6 (trial 2), fed chopped sugar beets, showed a retarded evacuation due to subnormal food consumption. Asterisk (*) indicates last recovery of sclerotia.

are somewhat inconsistent, and the differences are probably not significant.

PEPSIN DIGESTION

To test the effect of pepsin digestion on the viability of sclerotia, groups of 50 sclerotia each were immersed for 1 to 48 hours in a synthetic digestive solution prepared by adding 0.5 percent of pepsin to 0.5 percent of HCl and incubating for 4 to 6 hours at 40° C. The original solution had a pH value of 2.0, which remained nearly constant during the trials.

After the stated intervals duplicate groups of 50 sclerotia each were removed from the solution. One group was washed in sterile water, immersed in $HgCl_2$ 1–1,000 for 45 seconds, and then washed in three changes of sterile water before being plated on potato-dextrose agar. The other group of sclerotia was washed in sterile water and then plated without the $HgCl_2$ treatment. The results (table 2) show clearly that germination of the unsterilized groups was not inhibited by exposures of less than 12 hours in the pepsin solution. Longer exposures reduced the percentage of germination.

TABLE 2.—*Germination of sclerotia, plated with and without previous sterilization, after exposure to pepsin digestive solution at 37° C. for various periods*

Time of exposure (hours)	Germination of 50 sclerotia plated without sterilization	Germination of 50 sclerotia after sterilization with HgCl₂ 1–1,000 for 45 seconds	Time of exposure (hours)	Germination of 50 sclerotia plated without sterilization	Germination of 50 sclerotia after sterilization with HgCl₂ 1–1,000 for 45 seconds
	Percent	*Percent*		*Percent*	*Percent*
0 (controls)	100	100	12	98	0
1	100	100	24	86	36
2	100	92	36	64	0
6	100	74	48	32	2

Although the germination of normal sclerotia was not influenced by surface sterilization with HgCl₂, sclerotia weakened by immersion of 12 hours or more in the digestive solution were almost completely prevented from germinating by the HgCl₂ treatment.

These results suggested that surface sterilization with HgCl₂ may have been partly responsible for the low percentage of germination of sclerotia recovered from sheep feces collected during trials 1 and 2. To test this hypothesis trial 3 was conducted in March 1935, with one sheep being fed 2,500 sclerotia produced on carrots in laboratory moisture chambers. These were mixed with a single feeding of dry concentrates and fed as in the first trial.

RESULTS OF TRIAL 3

Collections were made at 12-hour intervals, and the sclerotia recovered as in previous trials. Instead of being surface-sterilized, however, the sclerotia were plated without further treatment on moist, finely screened, unsterilized peat soil in Petri dishes and incubated at 30° C. After having been plated, the sclerotia were kept under observation for at least 5 days unless germination occurred within a shorter period. The rate of recovery shown in table 3 is comparable to that obtained in the previous trials. Of the 2,500 sclerotia consumed, 13.3 percent were recovered within 60 hours, and no whole sclerotia thereafter. Of the 333 sclerotia recovered, however, 50 or 15.0 percent germinated when tested by the revised method, whereas the control sclerotia showed 58 percent germination. The percentage of germination in this trial, being considerably higher than that obtained in the first two trials, suggests that the number of viable sclerotia evacuated by the animals in the first two trials may have been considerably larger than was indicated by the figures.

TABLE 3.—*Recovery and germination of sclerotia, plated without surface sterilization after passage through the digestive tract of a sheep, trial 3* [1]

Time after ingestion (hours)	Sclerotia recovered	Germinated		Time after ingestion (hours)	Sclerotia recovered	Germinated	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
12	77	30	39.0	60 [2]	5	0	0.0
24	162	18	11.2				
36	65	2	3.8	Total	333	50	15.0
48	24	0	.0				
				Controls [3]			58.0

[1] The animal consumed 2,500 sclerotia.
[2] No recoveries at 72, 84, 96, or 108 hours.
[3] 100 sclerotia, a representative sample of original sclerotia before feeding.

EXPERIMENTS WITH CATTLE

Approximately 15,000 sclerotia collected from infected sugar beets and mixed with barley, wheat bran, and chopped alfalfa hay were fed at a single feeding to a Jersey cow. Samples of feces were screened to determine the earliest evacuation of sclerotia. None was found at 12 hours, but at the end of 24 hours a few were recovered. During the next 12 hours 226 sclerotia (fig. 3) were evacuated, of which 16, or 7.1 percent germinated despite the fact that they were subjected to HgCl₂ surface sterilization before plating. During the next 5 days small samples of feces were analyzed and some sclerotia recovered each time, but with a gradual decrease in number. Beginning at 156 hours after the sclerotia were consumed, all the feces were examined during the next 4 days, but no sclerotia were recovered.
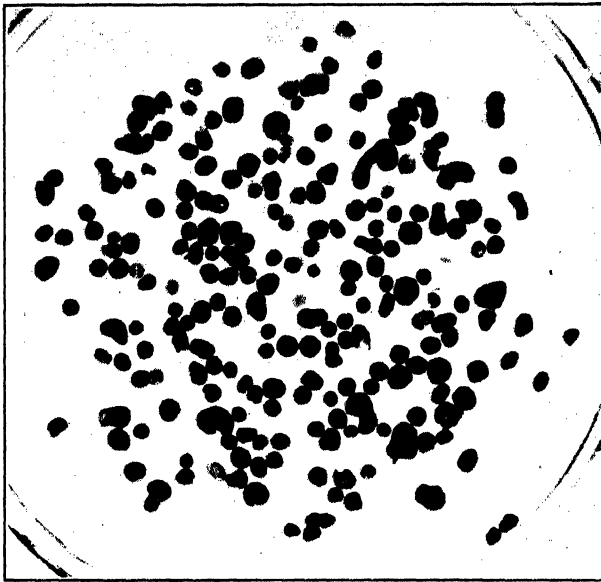
FIGURE 3.—Sclerotia of *Sclerotium rolfsii* recovered from a 12-hour collection of feces from a cow. Note that some have been broken by passage through the digestive tract. Of these 226 sclerotia, 16 (7.1 percent), germinated when plated on agar. × 1.6.

With this qualitative information in mind, another test was arranged to secure more definite information on the rate of passage, the length of retention in the body, and the percentage germination of scleortia recovered during each 12-hour period.

Approximately 14,500 field sclerotia, mixed with feed as in the preliminary trial, were offered to a second Jersey cow. Only a small portion of the feed, estimated to contain 3,000 sclerotia, was consumed at the first feeding; the remainder, containing about 11,500 sclerotia, was eaten 12 hours later. Each 12-hour collection of all feces was

thoroughly mixed, and aliquot samples consisting of 25 percent of the mass were taken for examination except when the weight exceeded 20 pounds, in which case 12.5 percent was taken. All sclerotia recovered were surface-sterilized with $HgCl_2$ and tested for germinability.

The number of sclerotia recovered, the approximate number evacuated, and the percentage of germination are presented in table 4.

TABLE 4.—*Recovery and germination of sclerotia after passage through the digestive tract of a cow*

Time after ingestion [1] (hours)	Sclerotia recovered	Germinated		Approximate number evacuated [2]	Time after ingestion [1] (hours)	Sclerotia recovered	Germinated		Approximate number evacuated [2]
	Number	Number	Percent			Number	Number	Percent	
12	1	0	0.0	4	132	5	0	0.0	20
24	85	21	24.7	340	144	1	0	.0	4
36	180	13	7.2	720	156	0			0
48	205	21	10.2	820	168	0			0
60	55	0	.0	220	180	1	0	.0	4
72	162	0	.0	648	192–228 (4 samples)	0			0
84	93	0	.0	744	Total	851	57	6.7	4,028
96	35	0	.0	280	Controls [3]			88.4	
108	19	2	10.5	152					
120	9	0	.0	72					

[1] Approximately 14,500 sclerotia were consumed, 20 percent of which were consumed at the first feeding and the remainder at the end of the first 12 hours.
[2] Obtained by multiplying the number of sclerotia recovered (column 2) by 4 or 8, according to the fractional part of the feces examined.
[3] 287 sclerotia, a representative sample of original sclerotia before feeding.

About 27.8 percent of the 14,500 sclerotia consumed were evacuated in a whole condition. Of those recovered, 6.7 percent germinated. On the other hand, 11.7 percent of the sclerotia recovered during the first 48 hours germinated, whereas only two viable sclerotia were recovered thereafter. At the time of these trials the deleterious effect of surface sterilization with $HgCl_2$ on weakened sclerotia was not known.

SUMMARY AND CONCLUSIONS

Sclerotia of *Sclerotium rolfsii* were added to the ration of seven sheep and two cows and their excreta examined for the presence and viability of sclerotia. From 8 to 28 percent of the total consumed were evacuated in a whole condition.

Examination of the contents of the digestive tract of two slaughtered sheep indicated that the remainder of the sclerotia were digested or fragmented, since no whole sclerotia were retained by the animals after a period of 5 days.

It was found that from 0.7 to 15.0 percent of the evacuated sclerotia retained their viability. No viable sclerotia were evacuated by sheep later than 84 hours and none by cattle later than 108 hours after ingestion.

Evidence was obtained that surface sterilization with $HgCl_2$ reduced the germination of weakened sclerotia, although sound sclerotia were not affected.

Under normal feeding conditions most of the living sclerotia were excreted within 48 hours after consumption. Subnormal food con sumption retarded the evacuation of sclerotia but did not prolong the period in which viable sclerotia were excreted.

Sclerotia immersed in the liquid contents of sheep rumen were still viable at the end of 48 hours. A pepsin digestive solution reduced but did not entirely prevent germination within the same period.

These investigations prove conclusively that sclerotia can pass through the digestive tract of cattle and sheep without complete loss of viability.

The total number of sclerotia that could be transported by the movement of animals would probably not be large as compared with the quantities now occurring in infested fields. Evidently, however, cattle and sheep may introduce into uninfested fields a sufficient quantity of the parasite to produce serious losses in future plantings.

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REPRODUCTIVE CAPACITY OF FEMALE RATS AS AFFECTED BY KINDS OF CARBOHYDRATES IN THE RATION [1]

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INTRODUCTION

When experimental rats used in a study of galactose metabolism [2] failed to reproduce normally, it was decided to determine the character of the abnormality and also, if possible, the relation of the carbohydrate in the ration to the reproductive capacity of the rat.

LITERATURE

The effects on reproduction of moderate amounts of sucrose and lactose in the rations of rats have been reported by several workers. Mitchell (6) [3] found that lactose, when constituting not more than 30 percent of the ration or 50 percent of the total carbohydrate in the ration, did not interfere with growth or well-being. Skinner, Van Donk, and Steenbock (8) found that ovarian function, as measured by length and regularity of oestrus cycles, was improved by the addition of 10 percent of sucrose to a ration of mineralized milk. Keil, Keil, and Nelson (4), however, reported interference with reproduction as a result of the addition of 5 percent of sucrose to a differently mineralized milk ration.

Evans (3) stated that a lack of vitamin E has no effect on the ova or ovarian tissue. In a comparison of vitamin E deficiency with that of vitamins A and B, he stated that vitamin A deficiency is characterized by a continuous cornified smear.

Smith and Engle (10) found that anterior pituitary transplants stimulated the ovaries of immature and mature rats, producing an excess of follicles. Ovulation occurred in the young animals. In older animals there were some follicular cysts, and there was also some luteinization. The cysts were sometimes twice the size of normal follicles. Engle and Smith (2), in a study of corpus luteum formation in pituitary-stimulated animals, found that follicles did not rupture, but formed cysts which transformed directly into corpora lutea. Sometimes eggs were found "trapped" as a result of luteinization of the follicular wall. Smith (9) observed that hypophysectomy of the rat resulted in a regression of the ovaries. If corpora lutea were present, they persisted for a long time. Transplants of anterior pituitary stimulated these regressed ovaries to renewed activity but follicular and lutein cysts resulted. Casida and Hellbaum (1), using 21- to 25-day-old rats, produced large follicles and

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corpora lutea by injections of extracts from adrenal glands of horses. They obtained ovulation in about 50 percent of the treated animals. They made no attempt to determine whether this effect was direct or indirect. Moore and Price (7) concluded from their work that the only gonad-stimulating hormones are hypophyseal or hypophyseallike substances.

MATERIALS AND METHODS

The rations fed were designed to be adequate in every known respect. One group of rations contained a minimum amount of galactose while the other group contained lactose which provided a liberal supply of readily available galactose.

The regular experimental rations (designated according to the characteristic carbohydrate) are shown in table 1.

TABLE 1.—*Composition of experimental rations*

Food product	Sucrose	Lactose	Starch
	Percent	*Percent*	*Percent*
Yeast	5	5	4
Osborne and Mendel salt mixture	4	4	5
Cod-liver oil	1	1	1
Casein	20	20	20
Butter oil	20	20	20
Starch	30	30	50
Sucrose	20		
Lactose		20	

Commercial lactose was used for all but one group of lactose-fed rats. The substitution of Mallinkrodt's "analytical reagent" lactose for this group produced no change in the results. No further attempt was made to differentiate lactose from factors which might be associated with it as the important constituent of the ration.

These rations were fed to many groups of rats during a period of more than 2 years. The early groups included several females for which the reproductive record is not complete enough to justify detailed description but which furnished the incentive for the detailed records set forth in this paper.

Some of the rats were mated in order to test fertility. Ages at which the vaginas opened were determined. Microscopic observations were made on the ovaries of some animals, and the uteri of these animals were examined macroscopically. Serial sections of the ovaries were made for microscopic study. In some cases one ovary and part of one horn of the uterus were removed by laparotomy. Since little difference was noted between the remaining ovaries of these animals and ovaries from other animals receiving the same feed, they have been included in the appropriate age and feed groups.

OBSERVATIONS

Seven animals fed lactose, five fed sucrose, and two fed starch were kept with fertile males for 40 days or until pregnancy was detected. During the time of this test all the animals were changed from the experimental rations to the regular stock ration. The results are presented in table 2.

TABLE 2.—*The effect of feeding various carbohydrates on the reproductive ability of rats*

| Animal no.[1] | Carbohydrate fed | Age of animal | | Size of litter | Condition of young |
		When placed with male	When litter was born		
		Days	*Days*	*Number*	
70a	Lactose	79	107	10	Normal.
78a	do	79	105	5	All died within 3 days.
84b	do	79	102	7	Normal.
90b	do	79	105	5	Do.
121c	do	56	80	4	1 died, 3 normal.
125c	do	56	93	8	Normal.
129d	do	48	85	8	Do.
1	Sucrose	80		0	
2	do	70		0	
3 [2]	do	70	111	3	2 died, 1 weakly.
87b	do	79		0	
119c	do	56	80		Abortion.
123c	Starch	56	94	10	Normal.
127c	do	48	87	7	Do.

[1] The animals having like letters were litter mates.
[2] After producing 1 litter, this female was kept with a fertile male for 90 days without producing another litter.

As table 2 shows, each lactose- and starch-fed animal reproduced. All these litters, except possibly one born to a lactose-fed female, were normal. The one sucrose-fed female which produced a live litter was afterward sterile.

The age at which the vagina opened and the type of vaginal smears were determined for 17 sucrose-, 4 starch-, 6 stock-, and 18 lactose-fed animals. The results are presented in table 3. The sucrose-fed

TABLE 3.—*Effect of feeding various carbohydrates on age at which vaginas of rats opened, and types of smears secured*

| Ration fed | Animals | Age at which vagina opened | | | Standard deviation | Vaginal smear record |
		Youngest	Oldest	Average		
	Number	*Days*	*Days*	*Days*		
Sucrose	17	32	51	38	4.8	1–3 cycles, then dioestrum.
Starch	4	45	53	49	3.8	Not observed.
Stock	6	51	60	55	3.4	Normal cycles.
Lactose	18	45	64	59	6.7	Do.

animals showed sexual activity, as measured by the age at which the vaginas opened, earlier than any of the other groups. There is considerably less than 1 chance per 1,000 that this difference is due to chance. The smears of the sucrose-fed animals, however, showed only a few oestrus cycles. The lactose-fed animals were slightly late in reaching sexual maturity but showed normal cyclic changes in the smears. The starch- and stock-fed groups consisted of only a few animals, but these showed no abnormalities. They closely resembled the lactose group.

The observations made on the ovaries of the animals fed sucrose and those fed lactose are shown according to age groups in table 4.

TABLE 4.—*Effect of feeding sucrose and lactose in relation to microscopic observations of the ovaries of rats in various age classes*

Animals (number)	Age	Carbohydrate fed	Corpora lutea	Luteinization of follicles	Atresia [1]
	Days				
3	37	Sucrose	+	+	−
2	37	do	−	+	−
1	37	Lactose	−	−	+
5	44-47	Sucrose	+	+	−
2	44-47	Lactose	−	−	+
10	64-72	Sucrose	+	+	−
10	64-72	Lactose	[2] +	−	+
6	64-72	do	−	−	+

[1] Atresia indicates that follicles are so degenerated that they should soon disappear.
[2] 3 animals in this group had fresh rupture points in some of the follicles. Eggs were found in the Fallopian tubes of 2.

Photomicrographs of typical sections from ovaries of sucrose- and lactose-fed animals of each age group are shown in plate 1, *A* to *F*. These sections all have the same magnification, but they do not represent the relative sizes of the ovaries from different animals.

The presence of luteinized follicles in the sucrose-fed animals of all age groups would account for the lack of atretic disappearance of follicles. Luteinization of immature follicles would also explain the failure of the follicles to rupture.

As table 4 shows, the sucrose-fed animals were precocious. Corpora lutea were found in the ovaries of some of these animals at 37 days of age (pl. 1, *A*). In sharp contrast to this, the lactose-fed animal of this age was practically in an infantile sexual condition. Small follicles only were present in the ovary (pl. 1, *B*).

At 44 to 47 days of age similar differences existed as in the 37-day-old animals but they were even more striking. The sucrose-fed animals showed many more corpora lutea, luteinization of the larger follicles, and only a few small follicles (pl. 1, *C*). There was some indication that the follicles were being transformed into corpora lutea without a rupture occurring; at least no rupture points were observed. Some of the large luteinized follicles contained eggs that were degenerating. The lactose-fed animals, on the other hand, contained ovaries with follicles of all sizes (pl. 1, *D*). There was no luteinization of follicles among lactose-fed animals.

In the age group 64 to 72 days, many large corpora lutea were found in the ovaries of the sucrose-fed animals. There were no cyclic ages of corpora lutea, and no small follicles were present. Some cysts due to luteinization of the large follicles were found, but none of the corpora lutea showed signs of degenerating as would be expected if normal cycles were occurring (pl. 1, *E*). There was considerable variation among the lactose-fed animals. Some had not yet ovulated, showing some indication of retardation. In most cases, however, fresh corpora lutea and normal follicles of different sizes were found (pl. 1, *F*). In some of the lactose-fed animals the rupture points were visible, and eggs were found in the tubes of two animals. More than one age of corpora lutea was observed in the ovaries of some animals. There was no luteinization of follicles.

A, Left ovary from a 37-day-old sucrose-fed rat, showing large corpora lutea (*cl*) and small luteinized follicles (*lf*). *B*, Left ovary from a 37-day-old lactose-fed rat, showing many small follicles (*f*). There is no luteinization of any follicles. Some atresia is present. *C*, Right ovary from the same animal as *A*, at 45 days of age. A sucrose-fed rat, showing large corpora lutea (*cl*) and luteinized follicles (*lf*). No normal-appearing follicles of any size. *D*, Right ovary from the same animal as *B*, at 45 days of age. A lactose-fed rat, showing follicles of different sizes (*f*). No corpora lutea and no luteinization of follicles. *E*, Ovary from 70-day-old sucrose-fed rat, showing many corpora lutea (*cl*) and luteinized follicles, some containing degenerating eggs (*de*). Some follicles are so heavily luteinized that cysts have resulted (*fc*). *F*, Ovary from 70-day-old lactose-fed rat, showing follicles of different sizes (*f*) and recent corpora lutea with blood clots (*rcl*). There is no luteinization of follicles.

The uteri of the sucrose-fed animals of all age groups were generally larger than those of the corresponding lactose-fed animals. Some were distended with fluid and some were highly vascular.

Ovaries of three lactose-fed animals were observed about 30 days after the animals had given birth to litters. The normal cyclic ages of corpora lutea were present. Ovaries of four other animals aged about 200 days were also observed. These had been on a sucrose ration until about 70 days of age. Afterward they had been on the regular stock ration. The ovaries were made up almost entirely of masses of corpora lutea and follicular cysts. No evidence of cycles was seen, and there was indication of the cysts transforming into corpora lutea.

DISCUSSION

With our present knowledge of nutrition it is difficult to understand how the replacement of either the 20 percent lactose, or 20 of the maximum 50 percent starch, with 20 percent sucrose in the ration could have produced the observed sexual precocity and luteinization of follicles. A few instances have been reported in which sucrose in the ration was associated with abnormal reproduction. The observed abnormalities, however, were not linked with either abnormal ovarian structure nor with any pituitary disturbances. The absence of galactose might possibly be associated with the results produced by the sucrose ration. Sorensen and Haugaard (*11*) have shown that casein contains a small amount of galactose. It is possible that the additional cornstarch contained sufficient galactose to meet the need and make possible the observed relations of the three rations studied. It might, on the other hand, be assumed that the sucrose affected some gland which secreted a gonad-stimulating substance. Since most work indicates that the pituitary is the only gland which could have produced this effect, there is the possibility that the sucrose either directly or indirectly affected the activity of the pituitary.

The results obtained were similar to those produced by pituitary implants (*10*). One difference, however, is that no excessive number of follicles or corpora lutea were present. The luteinization of follicles and formation of cysts and the persistence of the corpora lutea were more like the results produced by implanting pituitaries into hypophysectomized rats (*9*). The transformation of follicular cysts into corpora lutea is similar to results obtained by Engle and Smith (*2*). The similarity of the ovarian abnormalities in the writers' animals to defects noted by other workers when the pituitary function was altered is strong evidence that some pituitary disturbance existed.

The possibility that a vitamin deficiency might have brought about the abnormalities noted is very small. The symptoms observed were not the characteristic symptoms of any vitamin deficiency. The smear record and ovarian observations are far from indicating a deficiency of either vitamin A or E.

When the mixture of purified amino acids recently announced (*5*) becomes available as a substitute for proteins, it may be practical to determine more definitely what characteristics of the carbohydrates studied are associated with the results secured in this investigation.

SUMMARY

In this study of the relation of the carbohydrate in the ration to the reproductive capacity of rats, three carbohydrates were fed, sucrose, lactose, and starch.

The sucrose-containing ration was found to be inadequate for normal reproduction in rats, even when replaced during adult life by a normal ration.

Rats receiving the sucrose ration were precocious in sexual development.

Extreme luteinization of follicles and persistence of corpora lutea were characteristic of the ovaries of the sucrose-fed animals. This adequately explains the failure of these animals to reproduce.

The ovarian abnormalities of the sucrose-fed animals indicate pituitary disturbances.

Animals fed the lactose- and starch-containing rations reproduced normally and had normal ovarian structures.

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THE DISTRIBUTION AND CONDITION OF THE POTASSIUM IN A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN CYLINDERS [1]

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INTRODUCTION

In an earlier paper (19) [2] the writer reported the utilization and recovery of nitrogen, phosphorus, and potassium by apple trees grown in metal cylinders for a period of 6½ years. These trees received (each spring) during the last 3 years of growth different combinations of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate. The ratio in which nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) were absorbed from the added salts by the trees which gave optimum growth and reproduction, viz, those growing in the NPK- and NP-treated cylinders, was 3.0:0.3:1.5, whereas the ratio in which these same fertilizer entities were applied was 3:8:4. This latter ratio is based on current orchard practice. Because of the great divergence in these ratios studies were made to determine the distribution and condition of the nitrogen, phosphorus, and potassium in the soil at the end of the experiment. The results as they relate to nitrogen and phosphorus have already been reported (20, 21). The corresponding studies were made to determine the distribution and condition of the nitrogen, phosphorus, and potassium in the soil at the end of the experiment. The results as they relate to nitrogen and phosphorus have already been reported (20, 21). The corresponding data for potassium are given in the present paper.

Although both phosphorus and potassium are "fixed" by soils, the solubility relations of these elements are markedly different. The phosphorus concentration of the soil solution of a particular soil varies over a relatively small range, whereas that of potassium fluctuates over a wide range even when the soil neither receives applications of potassium salts nor suffers loss by leaching or removal by the plant.

In this paper all results have been calculated to and reported as the oxide K_2O, commonly designated potash. Occasionally, in the text, reference is made to the element potassium where the use of the ionic form appears to be the more suitable. The reader, however, should keep in mind that all values given in the tables are expressed as the oxide K_2O.

METHODS

THE FIELD PLAN

Since a detailed plan of this experiment has already been presented (1, 20, 21), only an outline of the procedure will be given here. A virgin soil (18) of Trenton formation, formed by the weathering of

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limestone, was used. This soil is designated throughout as the "original" soil, and represents the condition of the soil before the experiment began. The method of excavation, the preparation and mixing of each horizon, and the filling of the cylinders have been described in detail (*18, 20*) and also the method of sampling the soil before and after the experiment (*21*).

The sources of the potassium in this Hagerstown soil are the mixed feldspars, micas, and tourmalines. The silt fraction contains a preponderance of microcline with some quartz; whereas the fine sand (0.1 to 0.5 mm) consists of 50 percent of mixed feldspars and 35 percent of quartz. Orthoclase is present in very small amounts and is highly kaolinized. No fresh unaltered samples of orthoclase exist in this soil (*18*).

For the first 2 years after the trees were planted the culture system was uniform in all cylinders. It consisted of green manuring with buckwheat and rye principally. Commencing with the third year, the system was differentiated as follows: Half of the cylinders were kept under a system of clean cultivation. These cylinders are designated "cylinders under cultivation." Half the cylinders were seeded with a mixture of bluegrass and timothy. These cylinders are designated "cylinders under sod." The schedule of applications of potassium sulphate is given in table 1.

TABLE 1.—*Schedule of potassium sulphate applications with potash equivalent*

Date applied	K_2SO_4	K_2O equivalent
	Grams	*Grams*
Apr. 18, 1925	293	158.45
May 3, 1926	147	79.49
June 7, 1926	147	79.49
May 5, 1927	293	158.45
May 18, 1927	147	79.49
Total	1,027	555.37

The total amount of potash added in the fertilizer during the whole period of the experiment was, as indicated above, 555.4 g. In addition, 25 g of potash was added in the form of cover crops of all cylinders up to the period of differentiation referred to in the last paragraph. An additional 15 g of potash was added after this period, but to the cylinders under cultivation only. The purpose in making these relatively large applications of fertilizers was to saturate the fixing capacity of the 0- to 7-inch layer of the original virgin soil with respect to phosphoric acid. The amounts of nitrogen and potash applied were porportionally increased so as to maintain the ratio 3:8:4.

LABORATORY EXPERIMENTS

The method of approach in determining the condition of the potassium was to find, in addition to the total potassium, the amounts removed by continuous leaching with three solvents, namely, (1) freshly boiled distilled water, (2) M/2 ammonium acetate, and (3) 0.2 N nitric acid. A few experiments were also carried out with 1-percent citric acid, but these experiments are not recorded inasmuch as no additional information was obtained by this reagent.

The total potash (K_2O) was determined in the three horizons of all soils by the J. Lawrence Smith method *(22)*, a 0.5 g charge of finely pulverized soil being used. The detailed results for total potash are not recorded for reasons which will be apparent later (p. 538).

The continuous leaching experiments were conducted on 10 g charges of soil in the same manner and with the same apparatus that was described and illustrated in a preceding paper *(21)*. The temperature of the laboratory during the experiments varied between 75° and 85° F. Preliminary experiments showed that the increase in the amounts of potash extracted per 2° rise in temperature was equivalent to 0.9 percent of that extracted at 75°. This is relatively a negligible increase. The rate of percolation was approximately 1 drop in 90 seconds.

The potash content of the leachates was determined by three methods. When the amounts were sufficiently large for gravimetric procedure the potassium was precipitated and weighed as the chloroplatinate *(2)* after the removal of silica, iron, alumina, calcium, and barium. When, however, the amount of potassium extracted reached values below 1 mg in 200 cc of the extract, recourse was had to the sodium cobaltinitrite method as devised by Drushel *(7, 8)* and modified by Morris *(14)*. Further improvements have been introduced by Hibbard *(11)*.

The determinations of such extremely low values of magnitude as were given in all the extracts with distilled water and with ammonium acetate after the first leachate were carried out by the colorimetric method of Schreiner and Failyer *(17, pp. 31–33)*.

Difficulty has been experienced by some investigators with the platinic chloride method, especially on the extracts from ammonium salts, which are usually very high in calcium. When this method is used it is necessary for the analyst to assure himself of the complete removal of all traces of calcium before proceeding to the precipitation of the mixed chloroplatinates. This may be accomplished by treating the sirupy mixed chloroplatinates with 15 to 20 cc of a solution of acidified alcohol. The acidified alcohol is prepared by passing washed hydrochloric acid gas into commercial 95-percent alcohol until the solution is two to three times normal in strength. The usual procedure is then followed *(2)*.

EXPERIMENTAL DATA

The total potash (K_2O) content of the three horizons of the original, check, NPK, NK, and PK soils was found to range between certain maximal and minimal values shown in the following tabulation:

Horizon	Range of K_2O, percent
Surface soils (0 to 7 inches)	3. 919 to 4. 007
Subsurface soils (7 to 21 inches)	4. 197 to 4. 205
Subsoils (21 to 53 inches)	4. 405 to 4. 410

The probable error of analyses may be gaged from 10 determinations made on the original surface soil before the experiment began. The results were 3.925 ± 0.005.

The results of the percolation experiments with water, M/2 ammonium acetate, and 0.2 N nitric acid are given in table 2.

TABLE 2.—*Potash (K₂O) in successive leachings with distilled water, M/2 ammonium acetate, and 0.2 N nitric acid, expressed in parts per million of dry soil and in grams*

DISTILLED WATER

Description of soil	\multicolumn{10}{c}{Potash in successive leaching indicated}	Total in first 3 leachings	Absolute amount in first 3 leachings	Total in all leachings	Absolute amount in all leachings
	1	2	3	4	5	6	7	8	9	10				
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*Grams*	*P.p.m.*	*Grams*
Soil before trees were planted:														
0-7 inches	41.4	17.2	10.4	9.8	10.0	7.2	6.0	5.2	5.0	5.0	69.0	40.4	117.2	68.6
7-21 inches	27.9	12.0	9.8	10.0	8.5	6.4	6.4	6.0	5.5	5.5	49.7	58.6	98.0	115.6
21-53 inches	26.2	11.4	10.2	9.3	9.1	7.5	6.2	6.0	4.0	4.0	47.8	128.6	93.9	252.6
Check (sod):														
0-7 inches	44.7	15.2	12.7	10.1	9.4	10.0	9.9	9.7	9.8	9.8	72.6	42.5	141.3	82.7
7-21 inches	33.4	11.4	9.7	10.0	9.8	9.2	9.4	10.0	8.9	9.0	54.5	64.3	120.8	142.5
21-53 inches	26.9	10.7	10.3	9.0	9.1	8.0	9.4	9.7	9.0	9.0	47.9	128.8	111.1	298.8
Check (cultivation):														
0-7 inches	51.7	18.9	12.4	10.2	11.0	9.2	9.2	9.5	9.5	9.5	83.0	48.6	151.1	88.4
7-21 inches	37.5	14.2	10.7	9.0	9.5	9.5	8.0	9.0	9.0	9.0	62.4	73.6	125.4	147.9
21-53 inches	32.1	12.7	9.2	8.5	9.4	9.0	9.5	9.0	8.5	8.5	54.0	145.2	116.4	313.1
NPK (sod):														
0-7 inches	190.0	42.6	32.6	21.3	15.7	10.9	9.8	10.2	10.4	9.8	265.2	155.2	353.3	206.7
7-21 inches	77.2	27.4	15.6	10.8	11.1	9.2	9.2	9.0	9.5	9.0	120.2	141.7	188.0	221.7
21-53 inches	27.9	14.9	10.7	9.5	9.5	10.0	9.0	9.0	9.5	9.0	53.5	143.9	119.0	320.1
NPK (cultivation):														
0-7 inches	201.0	62.4	24.6	12.4	10.9	11.0	9.0	8.4	9.0	9.0	288.0	168.5	357.7	209.3
7-21 inches	82.6	29.4	14.2	10.0	9.0	9.0	8.5	8.5	8.5	8.5	126.2	148.8	188.2	221.9
21-53 inches	29.0	13.6	10.4	9.1	10.0	9.5	8.5	9.0	9.0	9.0	53.0	142.5	118.1	317.7
PK (sod):														
0-7 inches	177.4	35.4	1 27.9								240.7	140.8		
7-21 inches	80.4	25.2	1 12.9								118.5	139.8		
21-53 inches	25.7	16.2	19.4								51.3	138.0		
PK (cultivation):														
0-7 inches	188.2	33.7	1 25.4								247.3	144.7		
7-21 inches	90.4	25.6	1 12.2								128.2	151.2		
21-53 inches	28.2	12.9	17.2								48.3	129.9		

M/2 AMMONIUM ACETATE

Description of soil	1	2	3	4	5	6	7	8	9	10	Total in first 3 leachings	Absolute amount in first 3 leachings	Total in all leachings	Absolute amount in all leachings
Soil before trees were planted:														
0-7 inches	73.6	17.4	15.5	10.2	8.0						106.5	62.3	124.7	73.0
7-21 inches	101.8	35.3	17.9	12.4	8.5						154.5	182.2	175.4	206.8
21-53 inches	160.8	51.0	22.4	11.2	9.0						234.2	629.9	254.4	684.3
Check (sod):														
0-7 inches	106.1	34.8	17.4	12.1	9.5						158.3	92.6	179.9	105.3
7-21 inches	106.0	50.3	29.6	13.4	9.0						185.9	219.2	208.3	245.6
21-53 inches	172.9	44.5	22.6	12.8	8.2						240.0	645.5	261.0	702.0

	1	2	3	4	5	6	7	8	9	10		A	B	C	D
Check (cultivation):															
0–7 inches	112.2	37.4	16.2	12.0	8.5							165.8	97.0	186.3	109.0
7–21 inches	116.5	52.4	28.2	14.2	9.0							197.1	232.4	220.3	259.8
21–53 inches	160.5	51.8	22.7	10.4	8.8							235.0	632.1	254.2	683.7
NPK (sod):															
0–7 inches	329.4	51.4	24.3	12.2	9.4							405.1	237.0	426.7	249.7
7–21 inches	204.8	51.2	24.9	11.8	9.0							280.9	331.3	301.7	335.8
21–53 inches	167.9	48.4	23.2	12.4	8.0							239.5	644.2	259.9	699.1
NPK (cultivation):															
0–7 inches	322.4	50.2	30.4	12.1	10.0							403.0	235.8	425.1	248.7
7–21 inches	219.2	53.7	27.8	11.0	10.2							300.7	354.6	321.9	379.6
21–53 inches	163.8	49.6	24.9	10.2	9.5							238.3	641.0	288.0	694.0
PK (sod):															
0–7 inches	342.4	60.4	28.2									431.0	252.2		
7–21 inches	200.8	47.2	22.8									270.8	319.4		
21–53 inches	168.2	50.4	26.4									245.0	659.0		
PK (cultivation):															
0–7 inches	319.6	58.2	39.4									417.2	244.1		
7–21 inches	210.4	52.4	29.2									292.0	344.4		
21–53 inches	162.9	45.6	23.4									231.9	623.8		

0.2 N NITRIC ACID

	1	2	3	4	5	6	7	8	9	10		A	B	C	D
Soil before trees were planted:															
0–7 inches	196.3	104.7	72.4	53.9	37.4	29.6	25.4	23.2	22.4	20.6		373.4	218.5	585.9	342.8
7–21 inches	235.5	182.1	170.5	135.6	117.2	83.4	48.9	39.7	28.6	25.0		588.1	693.6	1,066.5	1,257.8
21–53 inches	347.8	213.1	187.9	168.6	149.2	92.1	70.6	49.4	37.4	30.6		748.8	2,014.1	1,346.7	3,622.3
Check (sod):															
0–7 inches	360.4	251.9	224.8	143.4	135.6	116.2	89.1	54.2	38.7	33.8		837.1	489.8	1,448.1	847.3
7–21 inches	392.6	203.4	156.9	143.3	120.6	112.4	91.1	42.6	39.7	35.4		752.9	887.9	1,338.0	1,577.9
21–53 inches	372.1	203.5	172.4	158.4	136.8	101.4	81.6	53.2	38.9	35.6		748.0	2,012.0	1,353.9	3,641.7
Check (cultivation):															
0–7 inches	347.3	262.6	219.8	144.6	130.8	119.7	88.6	52.4	39.6	38.4		829.7	485.5	1,443.8	844.8
7–21 inches	400.0	224.8	158.9	140.4	118.7	104.2	99.7	59.4	48.2	41.4		783.7	924.2	1,395.7	1,646.0
21–53 inches	370.4	200.6	158.4	150.2	144.5	102.4	81.4	71.8	52.6	40.9		729.4	1,961.9	1,373.2	3,693.6
NPK (sod):															
0–7 inches	830.0	244.2	235.9	135.0	96.9	88.4	77.5	61.0	42.1	38.4		1,310.1	766.6	1,849.4	1,082.1
7–21 inches	446.8	262.8	160.4	149.8	120.0	110.7	98.2	50.6	48.1	40.4		870.0	1,026.0	1,487.8	1,754.6
21–53 inches	382.2	208.4	178.4	172.8	137.8	112.4	91.4	63.8	58.9	42.6		769.0	2,068.4	1,448.7	3,896.7
NPK (cultivation):															
0–7 inches	817.4	239.4	220.6									1,277.4	747.4		
7–21 inches	459.2	260.4	179.4									899.0	1,060.2		
21–53 inches	370.6	208.0	190.4									769.0	2,068.4		
PK (sod):															
0–7 inches	848.8	147.2	135.6	116.2	100.6	93.0	77.2	60.4	40.1			1,131.6	662.1	1,735.3	1,015.4
7–21 inches	447.2	260.4	162.8	160.4	119.0	104.6	70.4	58.4	40.4			870.4	1,026.5	1,517.8	1,789.9
21–53 inches	383.8	208.4	178.6	166.4	138.2	109.4	70.4	42.8	31.4			770.8	2,073.3	1,422.2	3,825.4
PK (cultivation):															
0–7 inches	839.4	154.2	[1]141.4									1,135.0	664.1		
7–21 inches	462.8	278.0	[1]190.4									931.2	1,098.2		
21–53 inches	380.8	202.2	[1]168.4									751.4	2,110.7		

[1] Percolation experiments were not carried further.

ANALYSIS AND DISCUSSION OF RESULTS

DOWNWARD MOVEMENT OF POTASSIUM

As Determined From Data for Total K_2O

The error involved in the determination of total potash, although relatively small, is absolutely large when multiplied by the weights of the soil in each horizon, viz, surface 1,290 pounds, subsurface 2,600 pounds, and subsoil 5,930 pounds. When the data for the total potash from each horizon were analyzed it was found that the error of analysis was too great to permit conclusions to be drawn with respect to the movement of potassium applied as fertilizer.

As Determined From Percolation Data

More accurate information should be obtained from extraction or percolation data. The amount of potassium which has moved from one horizon to another can be obtained by the difference between the amounts removed by the respective solvents from corresponding horizons of treated and check cylinders. The data so obtained are shown in table 3.

TABLE 3.— *Increase in the amounts of K_2O removed in 3 leachings by distilled water, M/2 ammonium acetate, and 0.2 N nitric acid from the NPK and PK cylinders above that of the corresponding horizons of the check cylinders*

Description of soil	Distilled water	M/2 ammonium acetate	0.2 N nitric acid
	Grams	*Grams*	*Grams*
NPK (sod):			
0–7 inches	113	144	277
7–21 inches	77	108	138
21–53 inches	6	— [1] 5	56
NPK (cultivation):			
0–7 inches	120	139	262
7–21 inches	75	127	136
21–53 inches	— [1] 1	9	53
PK (sod):			
0–7 inches	98	160	172
7–21 inches	76	96	139
21–53 inches	3	17	61
PK (cultivation):			
0–7 inches	98	147	180
7–21 inches	77	117	174
21–53 inches	— [1] 6	— [1] 8	60

[1] Decrease.

The progressive increases in these values (table 3) as one proceeds from the distilled water, M/2 ammonium acetate, and 0.2 N nitric acid extracts in the 7- to 21-inch and 21- to 53-inch horizons of the respective treatments indicate the condition with respect to solubility, in each solvent, of the applied potassium sulphate that has moved downward. The quantities of potassium that have so moved are expressed as a percentage of the residual applied potash in table 4. The residual amounts can be calculated from the known amounts of potash applied and that absorbed by the trees (*19*).

The subsurface data show that amounts ranging from 14.1 to 15.3 percent of the portion of the residual applied potash which has moved into the 7- to 21-inch layer are soluble in water; that amounts ranging from 17.9 to 26.4 percent are removed by M/2 ammonium acetate and 27.1 to 32.1 percent by 0.2 N nitric acid.

TABLE 4.—*Fraction of the applied potash found in the different leachates from the subsurface and subsoil horizons expressed as a percentage of the residual applied potash*

Horizon and treatment	Distilled H₂O	M/2 NH₄.C₂H₃O₂	0.2 N HNO₃
Subsurface:	*Percent*	*Percent*	*Percent*
NPK (sod)	15. 1	21. 0	27. 1
NPK (cultivation)	15. 3	26. 4	28. 3
PK (sod)	14. 1	17. 9	25. 5
PK (cultivation)	14. 2	21. 6	32. 1
Subsoil:			
NPK (sod)	1. 2		11. 0
NPK (cultivation)		1. 9	11. 0
PK (sod)	. 5	3. 2	11. 4
PK (cultivation)			11. 0

The subsoil data are more difficult to interpret. The percentages of the residual potash removed by distilled water and M/2 ammonium acetate probably lie within the limits of error of the experiment. The high results for the amounts removed by 0.2 N nitric acid therefore may not indicate a downward movement into this horizon, but may be a reflection of the increased solubility in this solvent of the native soil potash as the result of the effect of the far greater root systems in the treated cylinders.

CONDITION OF THE POTASSIUM

THE WATER-SOLUBLE POTASSIUM

Since distilled water will remove the salts that have not been adsorbed by the clay and humus complexes of the soil, leaching with this solvent in in effect similar to that of leaching with an electrolyte. Brönsted's investigations (*4, 5*) indicate the character of solubility changes in the presence of electrolytes.

The concentration of potassium, which is relatively high in all of the first percolates with distilled water, rapidly falls in the second extracts, and the decrease is then more gradual as the electrolytes of the soil solution are removed. This gradual removal of electrolytes explains why the fraction of the so-called exchangeable potassium (p. — and table 5) removed by distilled water prior to the establishment of a constant concentration is so large.

TABLE 5.—*The quantities of so-called exchangeable potash removed in the first 3 leachings with distilled water*

Description of soil	Original soil	Check		NPK		PK	
		Sod	Cultivation	Sod	Cultivation	Sod	Cultivation
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Surface	64. 8	45. 9	50. 1	65. 5	71. 5	55. 8	59. 3
Subsurface	32. 2	29. 3	31. 7	42. 8	42. 0	43. 8	43. 9
Subsoil	20. 4	19. 9	23. 0	22. 3	22. 2	20. 9	20. 8

The results show how impossible it is to determine the real quantities of water-soluble and exchangeable potassium. The water-soluble potassium must necessarily contain a certain amount of exchangeable potassium, because the leaching with water of these

soils containing relatively large amounts (p. 534) of applied potassium sulphate is in fact equivalent to leaching with a dilute solution of a neutral salt—in this case potassium sulphate—containing no ion in common with the salt used to determine the replaceable potash, viz, ammonium acetate. This phenomenon is further discussed in the next two sections from the point of view of the more general aspect of soil problems. Moreover, the ammonium acetate leachates will necessarily contain the water-soluble potash. It is, therefore, obvious that the "replaceable" potash cannot be definitely and exactly determined.

The very large percentages of the so-called exchangeable potash removed by water in these experiments is consequently explicable. Thus, from 46 to 50 percent of the so-called exchangeable potassium (p. 541) is removed by distilled water in the first three extracts from the surface soil of the check cylinders and from 65 to 71 percent from the NPK cylinders. Even in the surface layer of the original soil 65 percent of the exchangeable potassium is removed by water in the first three extracts. In the lowest horizons the fraction of the exchangeable potassium removed in the first three extracts is reduced to approximately 20 percent.

In the check and all treated cylinders the concentration of potassium in the eighth and succeeding leachates with distilled water is reduced to approximately 10 parts per million of the dry weight of the soil. In the original soil the concentration tends toward a relatively constant level of about 5 parts per million as leaching is continued. These differences in the constant level reached by the original soil and in the soils in the cylinders at the end of the experiment are significant. The stage reached by reduction to the constant level is indicative of the existence of a potassium combination of such low solubility that the amount going into solution at each extraction is independent of the mass present in the soil. The influences to which both the check and treated cylinders have been subjected, therefore, have resulted in an alteration in the character of this insoluble potassium combination in the direction of increasing solubility.

The question may now be asked, What significance do aqueous extracts of soils possess with respect to potassium content? Water extracts of soils obtained by displacement, 1:5 water extraction, and percolation methods have played a considerable role in investigations relating to the potassium-supplying power of soils. But, as already indicated (p. 539), the significance of the results needs to be reconsidered in the light of recent acquisitions to our knowledge of the behavior of the adsorbed materials in the presence of electrolytes of which, in soils, calcium is such a dominant factor. Although, as in these experiments, small amounts of potassium are dissolved for long periods of continuous leaching of a soil, this fact affords no indication that the difficultly soluble combinations of potassium would be removed by water in the absence of all traces of electrolytes. A low but effective concentration in electrolytes may be produced by several processes, such as (1) action of the micro-organisms (the soil was not sterile in these leaching experiments), (2) the effect of humic acids which can effect a repartition between the elements adsorbed by the humus-clay complexes and the solution phase in contact with it (6), and (3) the effect of the slow solubility of carbonates and of silicic

acids in water. Under natural field conditions the potassium fixed by the soil can enter into solution only as the electrolytes are replenished by the processes cited. It follows that the amount of potassium removed in aqueous extraction by any method whatsoever is indicative not only of the amount of "soluble" potassium but also of its temporary content of nitrates, chlorides, bicarbonates, sulphates, etc.

This effect of electrolytes on the solubility of adsorbed potassium is striking. The relationship quantitatively demonstrated by Wiegner and Müller (*23*) with permutite and with six soil types by Barbier (*3*) is, when the exchangeable hydrogen ions are relatively small compared with calcium

$$\frac{\frac{P}{C}}{1+\frac{P}{C}}=\frac{k}{T}\cdot\left(\frac{p}{c}\right)^{\frac{1}{n}}$$

where P and C are the concentrations of potassium and calcium in the adsorbent, p and c the concentration of these elements in the solution, k and n constants, and T the exchange capacity.

This formula shows that at equilibrium the ratio of potassium to calcium fixed in the adsorbing complex determines the ratio of potassium to calcium in solution. Accordingly, for one and the same concentration of potassium in the adsorbing complex at equilibrium, the concentration of potassium in the aqueous phase is never constant as the classical theory of adsorption holds. On the contrary, it is proportional to the final concentration of other cations, of which calcium, because of its relatively greater proportion to other cations in soils, plays the dominant role, and consequently, the concentration of potassium is proportional to the sum p plus c of potassium and calcium dissolved. This sum is equal to the quantity of anions present in the aqueous phase, i. e., to the total salt concentration of the solution. The concentration of the soil solution in potassium is then an increasing function of two factors (1) the ratio $\frac{P}{C}$ between the potassium and calcium "fixed" at equilibrium, which determines the ratio $\frac{p}{c}$ between potassium and calcium dissolved, and (2) the total salt concentration of the solution.

An appreciation of the significance of these facts will explain the difficulties encountered in all attempts to obtain threshold values for soil potassium; and, furthermore, will suggest the direction in which investigations on availability of soil potassium should proceed.

THE SO-CALLED EXCHANGEABLE POTASSIUM

It follows from Wiegner's observations discussed above that the so-called exchangeable potassium is, subject to the limitations of definition of the term "exchangeable", a variable quantity determined by the conditions of the experiment. For the determination of this so-called exchangeable soil potassium, extraction with a solution of either sodium or ammonium acetate has certain advantages over other reagents (*16*). In the present experiments a half-molar solution of ammonium acetate was used. This strength is sufficiently high, especially for percolation methods. Even in one extraction

Barbier (*3*) found that a normal solution of ammonium chloride displaced the whole, and a one-third normal solution 98 percent, of the exchangeable potassium from clay soils.

The results given in table 2 show that under the conditions of continuous leaching adopted in these experiments three extractions with M/2 ammonium acetate were necessary before the concentration of potassium was reduced to that of the constant level reached by the water leachates in the fourth extraction. After the third percolation the amounts of potassium removed by M/2 ammonium acetate were nearly identical for all the soils of this experiment. The addition of the potassium sulphate fertilizer resulted in an increase of the so-called exchangeable potassium of the surface horizons of the treated cylinders approximately two and a half times above that of the check cylinders. The effect of the cover crops on the exchangeable potassium was also quite marked. This is indicated by increases of 50 and 75 percent in the surface and subsurface soils of the check cylinders, respectively, over the corresponding horizons of the original soil.

The residual quantities of potash shown in column 2 of table 6 can be calculated from the amounts of potassium sulphate applied together with that absorbed by the trees from the added potassium sulphate (*19*).

TABLE 6.—*Quantities of residual potash which have accumulated in the exchangeable form in the 0- to 21-inch layer*

Description of soil	Residual K_2O (x)	Excess of exchangeable K_2O of treated cylinders over that of check cylinders (y)	$\frac{y}{x} \times 100$
	Grams	*Grams*	*Percent*
NPK (sod)	508	253	50
NPK (cultivation)	481	266	55
PK (sod)	536	256	48
PK (cultivation)	541	264	49

The differences shown in table 6 between the exchangeable K_2O of the treated cylinders and the corresponding check cylinders of the 0- to 21-inch horizon indicate that about one-half of the potassium applied to the soils may be considered to be in a replaceable form. Actually the amounts must be somewhat lower (p. 541). The remainder, consequently, must have been transformed into a nonexchangeable form and the greater portion in the 0- to 7-inch horizon.

The possibility of the conversion of surface-adsorbed exchangeable bases into a nonexchangeable form and vice versa is suggested by the results of several investigators (*10, 12, 13, 15*). This experiment supplies additional and possibly less vulnerable evidence of the fact.

RELATIVE AMOUNTS OF RESIDUAL APPLIED POTASH REMOVED BY THE DIFFERENT SOLVENTS FROM THE 0- TO 21-INCH LAYER OF THE NPK CYLINDERS

The relative amounts of residual applied potash removed by the different solvents (total of all leachings in each case) can be determined in a manner similar to that used to determine the phosphoric acid removed by various solvents (*21*).

Table 7 gives the potash removed by the different solvents from the 0- to 21-inch horizon of the NPK cylinders expressed as a percentage of the residual applied potash. The amounts of potash dissolved by the unfertilized (check) soils by the respective solvents are subtracted from the quantities removed from the treated cylinders as shown in column 2, under the indicated method of calculation.

TABLE 7.—*Relative magnitudes of the potash removed by the different solvents from the 0- to 21-inch horizon of the NPK cylinders*

IN SOD

Solvent	Indicated method of calculation	Percentage of residual applied K_2O dissolved
	Grams	
Distilled H_2O	$\frac{428.4-225.2}{508}\times100=$	40.0
$M/2$ $NH_4.C_2H_3O_2$	$\frac{605.5-350.9}{508}\times100=$	50.1
0.2 N HNO_3	$\frac{2,836.7-2,425.2}{508}\times100=$	81.0

IN CULTIVATION

Distilled H_2O	$\frac{431.2-236.3}{481}\times100=$	40.5
$M/2$ $NH_4.C_2H_3O_2$	$\frac{628.3-368.8}{481}\times100=$	53.9

Distilled water has removed 40 percent; M/2 ammonium acetate 50 to 54 percent, and 0.2 N nitric acid 81 percent, respectively, of the residual potash of the 0- to 21-inch layer. The relatively large percentages removed by distilled water as compared with that removed by M/2 ammonium acetate is related to the phenomenon already fully discussed (p. 541).

Eighty-one percent of the residual applied potash has been removed by 0.2 N nitric acid from the 0- to 21-inch horizon of the NPK cylinder in sod. These values give the relative condition of availability of the potash applied as the fertilizer potassium sulphate.

The potash removed by 0.2 N nitric acid has been designated by Fraps (9) as the "active" potash. It is surprising that the exchangeable potash of many soils reported by Fraps is from two to three times the amounts found by him to be soluble in 0.2 N nitric acid. In these cylinder experiments the potash dissolved from the corresponding horizons of the respective cylinders by 0.2 N nitric acid are from two to three times the values for the exchangeable potash even in the first two extractions alone. If the analyses are comparable with those here reported there can be no relation between the replaceable potash and that extracted by 0.2 N nitric acid from different soil types.

THEORETICAL FERTILIZER RATIO OF N:P_2O_5:K_2O FOR APPLE TREES GROWING ON A HAGERSTOWN CLAY LOAM SOIL

It was pointed out in the introduction to this and the two preceding papers of this series (*20, 21*) that the ratio in which nitrogen, phosphoric acid, and potash were absorbed by the NPK- and NP-

treated trees was 3.0:0.3:1.5, whereas the ratio in which these same fertilizer entities were applied was 3:8:4. The divergence between these ratios is great.

Certain contemporary German agronomists and horticulturists have sought to determine the fertilizer requirements of crops by modification of the quantities in which the principal fertilizer entities are found in the mature crop considered, by making allowance for the quantities of nitrogen leached and the phosphoric acid and potash fixed by the particular soil used.

The factors most frequently used by the German investigators have been for nitrogen, 0.33; for phosphoric acid, 0.50; and for potash, 0.25. The amount of each of these entities found in the mature crop is then multiplied by the proper factor and the resulting values are added to the amount of each entity in the crop.

Since the quantity of nitrogen in plants is roughly proportional to the supply, this is a rational procedure for determining the nitrogen requirement; but its applicability in determining the amounts of phosphoric acid and potash required is more limited because, owing to fixation, the amounts of these entities absorbed by the plant are not proportional to the supply. In a previous paper of this series (*21*) it was shown that the fixing capacity of any one soil type is a relative and not an absolute property; the amount retained is conditioned not only by the concentration of the phosphate or potassium ions but also by the concentration of other ions, such as hydrogen, silica, iron, aluminum, calcium, magnesium, etc. It is also obvious that the amounts fixed will depend upon the manner of application. By a large localized application of a phosphate or potash fertilizer, the soil in that immediate neighborhood may be completely saturated. It has also been shown in a preceding paper (*21*) that the iron and aluminum of the original soil was only 66 percent saturated with respect to phosphoric acid. At the end of the cylinder experiments, with a residual applied phosphoric acid and potash content of 1,040 and 508 g respectively, one-third and one-fifth, respectively, of this residual phosphoric acid and potash of the NPK cylinders under sod were transformed into very insoluble forms.

The proper values for nitrogen, phosphoric acid, and potash to be used in modifying the amounts absorbed by the trees would therefore become:

Nitrogen leached _____ $0.33 \times 152.3 = 50.2$ g
Phosphoric acid fixed_____ $0.33 \times 1,040 = 343.2$ g
Potash fixed_____ $0.20 \times 508 = 101.6$ g

Therefore, the theoretical quantities for application to the NPK cylinder under sod taken as an illustration would be:

Nitrogen_____ $152.3 + 50.2 = 202.5$ g
Phosphoric acid_____ $19.2 + 343.2 = 362.4$ g
Potash_____ $47.2 + 101.6 = 148.8$ g

where 152.3, 19.2, and 47.2 are the amounts of nitrogen, phosphoric acid, and potash absorbed by the NPK tree in sod from the added salts (*21*). The theoretical ratio thus derived is 202.5:362.4:148.8, or a 4.47:8.0:3.28 fertilizer. This compares with a ratio used in this experiment based on results in the experimental orchard of 3:8:4.

The reason, therefore, why the ratios in which the nitrogen, phosphoric acid, and potash absorbed by the trees differs so greatly from both the theoretical and "practical" ratios is now apparent.

SUMMARY

The distribution and condition of potash at the end of an experiment lasting 6½ years is given for three horizons of a Hagerstown clay loam soil contained in cylinders planted to apple trees and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate in different combinations.

Approximately one-third of the residual applied potassium has moved into the subsurface (7-to 21-inch) layer. There is no definite evidence of movement of applied potassium into the 21- to 53-inch layer.

Under the conditions of continuous leaching of these experiments the amounts of potash removed from the surface soils of the check and also of the treated cylinders by distilled water was more than half of that removed by M/2 ammonium acetate (the so-called exchangeable potash).

The necessity for the reconsideration of the significance of all water-soluble and so-called exchangeable potash data in soil investigations is discussed.

The system of green manuring adopted in this experiment has resulted in increasing the so-called exchangeable potassium of the 0- to 21-inch layer almost 50 percent.

Only about 50 percent of the residual applied potassium was found in a form soluble in M/2 ammonium acetate. The remainder has been converted into less soluble forms.

The relative quantities of the residual applied potash removed under conditions of continuous percolation from the 0- to 21-inch layer of the NPK-treated cylinder in sod by distilled water, M/2 ammonium acetate, and 0.2 N nitric acid were 40, 50, and 80 percent, respectively.

From the present and earlier reported experiments a theoretical fertilizer ratio for apple trees in a Hagerstown clay loam soil is derived. The theoretical ratio of $N:P_2O_5:K_2O$ is 4.47:8.0:3.28.

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FACTORS AFFECTING THE VITAMIN C CONTENT OF APPLES [1]

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INTRODUCTION

Marked variations in the vitamin C content of different varieties
of apples have been observed. The values of 21 Massachusetts-grown
varieties were reported by Smith and Fellers,[2] and the results of
several other workers have been summarized by Batchelder.[3] Be-
tween different lots of apples of the same variety small differences in
vitamin C concentration have been found which appeared to result
from the kind of fertilizers used (Potter and Overholser).[4] Nelson
and Mottern [5] report that the vitamin C content of oranges is low in
fruit from trees that have been heavily sprayed with lead arsenate.
It seemed possible that the adverse effect of the spraying might
have resulted primarily from a lowering of the effective photo-
synthetic capacity of the leaf.

A change in the ratio of leaf area to fruit, effected by thinning of
leaves to produce a low ratio and by thinning of undeveloped fruit to
produce a high ratio, would afford a means of measuring directly the
influence upon the vitamin C content of fruit of a change in its
supply of the products of photosynthesis. Hence, in this investiga-
tion of the factors that affect the vitamin C content of apples, a study
was made of the concentration of this vitamin in apples grown with a
high ratio of leaf area to fruit as compared with apples grown with a
low ratio of leaf area to fruit. A study was also made of size of in-
dividual apples as a factor in the measurement of the vitamin C con-
tent. Two varieties of apple, Delicious and Winesap, were used.
Both varieties were grown with different ratios of leaf area to fruit in
connection with the experimental work of the Division of Horticulture
of the Washington Agricultural Experiment Station at Pullman.
Each variety was grown under uniform conditions of fertilization,
spraying, and other environmental factors, the sole variant being
ratio of leaf area to fruit.

METHODS

Delicious apples of mature trees grown on large, unringed branches
were adjusted the first week in July, in one case to an approximate
leaf-fruit ratio of 60 leaves to each fruit and in another case to 20

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leaves to each fruit. Winesap apples were grown with similar leaf-fruit ratios, but upon ringed branches.

The apples were stored at 32° F. soon after harvesting, since results obtained the previous season with Delicious apples [6] had indicated that vitamin C is almost completely preserved at this temperature for the length of time covered by these experiments.

The method of vitamin C determination was similar to that hitherto employed in this laboratory, being a modification of the method of Sherman, La Mer, and Campbell.[7] In the first series of experiments, the apples were fed in radial sections.

RESULTS

VITAMIN C CONTENT AS AFFECTED BY RATIO OF LEAF AREA TO FRUIT

The results of the first series of experiments, with Delicious apples, are presented in table 1. Almost no difference was observed between the Delicious apples grown at the high leaf-fruit ratio and those grown at the low. The results obtained with 20 g of Delicious apple in each case corresponded closely with results obtained in earlier work by Batchelder [8] with check Delicious apples at this level. Guinea pigs receiving 23 g showed very slight scurvy, while those receiving 25 g in the previous study were completely protected. Apparently 25 g of Delicious apple represented very nearly the minimum protective dose according to the methods employed, with apples grown under the conditions prevailing in the station orchard. Different results reported from other laboratories may possibly be attributable to differences in size of apples or method of sampling, as later shown.

TABLE 1.—*Antiscorbutic potency of radial sections of Delicious and Winesap apples grown with high and low leaf-fruit ratios when fed to guinea pigs as the sole source of vitamin C*

Vitamin C supplement	Animals	Average daily gain or loss in weight	Average scurvy score
Delicious, high leaf-fruit ratio:	*Number*	*Grams*	
23 g	4	3.8	2.0
20 g	5	2.0	4.4
17 g	5	2.3	6.4
Delicious, low leaf-fruit ratio:			
23 g	6	1.8	2.2
20 g	6	1.7	5.5
17 g	5	1.7	6.2
Winesap, high leaf-fruit ratio: 8 g	8	1.6	9.9
Winesap, low leaf-fruit ratio: 8 g	8	1.8	4.9
Cabbage, 20 g [1]	11	3.1	.5
None [2]	2	−3.7	20.0

[1] Positive controls.
[2] Negative controls.

Contrary to the results obtained with Delicious apples, there was considerable difference between the Winesap apples grown at the two leaf-fruit ratios, those grown at the low ratio having a higher vitamin

[6] BATCHELDER, E. L. See footnote 3.
[7] SHERMAN, H. C., LA MER, V. K., and CAMPBELL, H. L. THE QUANTITATIVE DETERMINATION OF THE ANTISCORBUTIC VITAMIN (VITAMIN C). Jour. Amer. Chem. Soc. 44: 165-172, illus. 1922.
[8] BATCHELDER, E. L. See footnote 3.

C content than those grown at the high ratio. These apparently conflicting results with Delicious and Winesap apples may be explained on the basis of size, since the high- and low-ratio Winesaps showed the marked difference in size usually observed between apples grown under very different conditions; whereas the Delicious apples, presumably because the thinning was not completed sufficiently early and because the apples with the adjusted leaf-fruit ratios were not grown on ringed branches, were nearly the same in size and color, and, according to analyses, similar in chemical composition throughout.

VITAMIN C CONTENT AS AFFECTED BY SIZE OF APPLE

The question why the smaller Winesaps (grown at a low leaf-fruit ratio) were richer in vitamin C than the larger Winesaps (grown at a high leaf-fruit ratio) then presented itself. Theoretically, the size of the apple may cause a considerable difference in the scurvy-preventing action of radial sections of apples, or of apples which have been cored and ground before sampling, because there will, in both cases, be a higher proportion of skin in samples from small apples than in samples from large apples; and the skin is known to be much richer than the pulp in vitamin C.[9] Further study of the possibility that the difference between the vitamin C content of high and low leaf-fruit ratio Winesap apples might result from difference in size alone (the smaller apples showing weight for weight the higher vitamin C content) seemed desirable since otherwise it would apparently be necessary to conclude that the difference between the two types of Winesap was in inverse proportion to the nourishment received, and that the similarity of the two types of Delicious apple resulted from the fact that they were thinned too late and not grown on ringed branches, and hence were actually alike in their nutritive history.

A second comparison was therefore made to determine the effect of size on the vitamin C content of apples as ordinarily sampled. Check Winesap apples, the largest and smallest of the lot, were selected for comparison. Table 2 shows the results of feeding 8-g radial sections of large and small Winesap apples. This level was chosen as being probably slightly below the minimum protective dose and, therefore, capable of disclosing slight differences in vitamin C concentration. The average scurvy score shown by guinea pigs fed 8-g radial sections of large Winesaps (average weight 141 g) was somewhat higher than that of animals fed 8-g radial sections of small Winesaps (average weight 79 g), indicating that the large Winesaps contained, weight for weight, less vitamin C than the small Winesaps. Several guinea pigs refused to eat their apples and only four could be used in averaging the results. While a larger number of tests would have been desirable, the difference both in degree of scurvy and in rate of growth was so consistent that it seems certain that further tests would have strengthened the conclusion that the vitamin C supplied by an 8-g radial section of a large Winesap apple is measurably smaller than that supplied by a similar section of a small apple from the same tree.

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[9] FELLERS, C. R., ISHAM, P. D., and SMITH G. G. VITAMIN C DISTRIBUTION IN BALDWIN AND M'INTOSH APPLES. Amer. Soc. Hort. Sci. Proc. (1932) 29: 93–97, illus. 1933.

TABLE 2.—*Antiscorbutic potency of large and of small Winesap apples; of sections containing peel and pulp from Winesap apples grown at high and at low leaf-fruit ratios, and of sections from Winesaps stored at 32° and at 40° F. for 6 months, when fed to guinea pigs as the sole source of vitamin C*

LARGE AND SMALL WINESAP APPLES; 8-G PORTIONS

Vitamin C supplement	Animals	Average daily weight gain	Average scurvy score
	Number	*Grams*	
Large Winesap	4	0.7	12
Small Winesap	4	1.6	9

SECTIONS CONTAINING PEEL PLUS ONE-FOURTH INCH OF UNDERLYING PULP FROM WINESAPS GROWN AT HIGH AND LOW LEAF-FRUIT RATIOS; 8-G PORTIONS

Winesap, high leaf-fruit ratio	5	1.1	5
Winesap, low leaf-fruit ratio	6	2.1	4

RADIAL SECTIONS OF WINESAPS STORED AT DIFFERENT TEMPERATURES FOR 6 MONTHS; 10-G PORTIONS

Winesaps stored at 32° F	5	1.5	6
Winesaps stored at 40° F	8	.3	11

VITAMIN C CONTENT AS AFFECTED BY RATIO OF SKIN TO PULP

A direct comparison of the vitamin C content of the skins of the two types of Winesaps presented difficulties in sampling because of the impracticability of completely removing adhering pulp. On the other hand, direct comparison of the pulps presented difficulties because of the large size of the sample required and the refusal of the guinea pigs to eat a sufficient quantity of the pulp. A method of sampling was therefore devised by which both difficulties were minimized. Table 2 gives the results of a comparison of high and low leaf-fruit ratio Winesaps sampled in such a way that the proportion of skin and pulp was the same for both the large and the small apples. The apples were so sampled that each section of skin had one-quarter inch of pulp attached. The curve of the outer skin was followed in cutting the pulp so that the resulting samples from large apples or from small apples consisted of pulp and skin in similar proportions. While variations in the amount of pulp included in each sample were not completely eliminated by this method of sampling, the variations represented a much smaller percentage of the total weight of the sample and caused a much smaller error in the apparent vitamin C content. The average weight of the high-ratio apples fed was 175 g, and that of the low-ratio apples 90 g. The average scurvy scores for animals fed 8 g of apple sampled as described above was five for the high-ratio and four for the low-ratio apples. These values indicated that, when ratio of skin to pulp was taken into account, the same concentration of vitamin C was present in the tissues of apples grown under widely different leaf-fruit ratios. For the reasons mentioned above, separate tests for pulp and skin were not attempted in connection with this study, but it may be inferred from the foregoing results that the concentration of vitamin C was similar for similar tissues of both large and small apples.

Table 2 also gives the vitamin C value of Winesap apples held at 32° and 40°F. for 6 months.   Apples of medium size (average weight 110 g.) were fed in 10-g radial sections.   Apples stored at 32° produced only mild scurvy (average score 6), while those stored at 40° produced moderate scurvy (average score 11).   Apparently the vitamin C content was better conserved at 32° than at 40°.   Comparable results for these apples before storage were not available so that definite conclusions cannot be drawn as to the loss, if any, of vitamin C during 6 months' storage at 32°.   Previous work, summarized by Batchelder,[10] indicated that 10 g of Winesap apples completely protected guinea pigs from scurvy.   But possible differences in the size of the apples, fertilization of the trees, and other factors make direct comparisons of the vitamin C value of the stored apples reported here with that found in previously reported experiments on freshly harvested Winesaps, inadvisable.

SUMMARY AND CONCLUSIONS

The vitamin C content of apples was studied with respect to the effect of (1) the ratio of leaf area to fruit; (2) the size of fruit as it affected the ratio of skin to pulp, by various methods of sampling; and (3) storage at 32° and 40° F. for 6 months.   It was concluded that the ratio of leaf area to fruit affected the vitamin C content of apples only indirectly, i. e., as it affected the size of the fruit produced; that the size of the fruit was an important consideration under common methods of sampling because the ratio of skin to pulp was higher in small apples than in large apples, and the skin contained a higher concentration of vitamin C than the pulp; that storage at 40° resulted in greater loss of vitamin C than did storage at 32°; and that the vitamin C content of Delicious apples in the present study corresponded closely with values hitherto reported from this laboratory.

[10] BATCHELDER, E. L. See footnote 3.

THE DETERMINATION OF THE APPARENT DIGESTIBILITY OF GREEN AND CURED GRASS BY MODIFIED PROCEDURES [1]

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INTRODUCTION

In 1926 Bergeim (*1*)[2] presented a simplified method for determining food digestibility and utilization that has certain advantages over the standard method. His method consists in adding iron oxide to the food and determining the ratio of the amount of a given food substance to the amount of iron in the feed and feces. The percentage utilization is calculated from these ratios. The ratio of a nutrient to iron in the feces when divided by the ratio of the nutrient to iron in the feed and multiplied by 100 gives the percentage of the nutrient not utilized. The percentage subtracted from 100 gives the percentage digested.

The following formula illustrates the method of computation:

$$100-\left(\frac{\text{Percent nutrient in feces}}{\text{Percent iron in feces}}\times\frac{\text{Percent iron in feed}}{\text{Percent nutrient in feed}}\right)100=$$
apparent digestibility.

Bergeim proposed the use of iron oxide as a key substance because it is not appreciably absorbed from the intestines and is excreted practically in its entirety in the feces.

Gallup (*2*) compared the method proposed by Bergeim with the usual method of determining the digestibility of protein, which consists in measuring the amount of protein consumed and the amount excreted in the feces for a given length of time. This comparison was made with rats fed cottonseed-meal products. The apparent digestibility obtained by the Bergeim method was found to be slightly lower than that obtained by the standard method. Gallup attributed the lower figures to the loss of iron as it passed through the alimentary tract.

Investigations by Heller, Breedlove, and Likely (*5*) indicate that the normal iron content of a ration is a more accurate key substance than added iron oxide because it insures a more uniform intake and a more evenly distributed iron excretion in the feces. They concluded from investigations with rats that results obtained by the Bergeim method are comparable to those obtained by the old method. Comparisons of the apparent digestibility of the various feed constitutents determined by the two methods show fluctuations in results for the iron method both above and below the results obtained by the standard method.

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Gallup (*3*), in determining the digestibility of protein with rats, used silica as a key substance in place of iron oxide. His experiments with silica showed a smaller variation from the usual method than when iron oxide was used.

Gallup and Kuhlman (*4*) conducted an experiment with yearling Jersey heifers to determine by modified methods the digestibility of protein in cottonseed meal. They found that the coefficients of digestibility for the protein in the rations fed agreed very closely with those obtained in previous experiments by the ordinary methods. These investigators found that naturally occurring silica in the feed "served as a better index of the digestibility of the other substances than did the iron which had been added for that purpose."

METHODS

Additional work was necessary to determine the applicability of this modified method to nutritional work with larger animals. In conjunction with a number of digestion experiments, iron and silica determinations were made and apparent digestibility was calculated by the modified procedure and compared with results obtained by the standard method. Naturally occurring silica and naturally occurring iron were used as the key substances. The first digestion experiments conducted were with three 2-year-old Holstein heifers. In the first experiment these heifers were fed solely on artificially dried pasture grass, and in the second, solely on dried apple pomace. These experiments were conducted in the usual manner as described in previous publications (*6*, *8*).

In addition, silica and iron determinations were made on the feed and on the feces. Under the conditions of these experiments, it was found that silica was unreliable as a reference substance because of the impracticability of preventing contamination of the feed and feces. The wind blows frequently and vigorously in the section in which the experiments were conducted and considerable dust gets into both feed and feces, thus making the silica content an unreliable criterion. Moreover, considerable dirt may be incorporated in the feed at the time of harvest. Methods for the determination of the small amounts of naturally occurring iron also were inadequate.

In a digestion experiment with pea straw, iron and silica determinations were made. The results of this experiment confirmed the findings of the previous trials both with regard to the contamination by dust and also the lack of accuracy of determinations of small amounts of iron by the method used.

Experiments were undertaken to determine the effect of the temperature of drying on the digestibility of artificially dried pasture grass. Three wether sheep were used and six experiments were conducted. The methods of experimentation and the results obtained by the standard method have been reported in a previous paper (*7*). Improvement in methods of determining small amounts of iron in the feed and feces through the use of a photoelectric scopeometer made possible accurate and consistent iron determinations in this series of experiments. Three sheep were used in each of five experiments and two in the other and digestibilities were calculated for dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract for each sheep in each experiment.

RESULTS

A comparison of the apparent digestibility of nutrients in the feeds as determined by the modified and the standard method is given in table 1. The apparent digestibility calculated by the modified method varied both above and below that obtained by the standard method. The average percentage variation in digestibility of the modified method from the standard is given, together with the ratio of the difference to its probable error. This ratio indicates the significance of the variation. The variation among the individual animals in each digestion experiment for each nutrient is not materially greater by the modified than by the standard method.

TABLE 1.—*Comparison of apparent digestibility of nutrients in green, sun-cured, and artificially dried grass when fed to sheep, as determined by the standard and the modified (iron) method*

Treatment of grass fed	Sheep no.	Crude protein			Crude fiber			Ether extract			Nitrogen-free extract		
		Standard method	Modified method	Difference	Standard method	Modified method	Difference	Standard method	Modified method	Difference	Standard method	Modified method	Difference
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Green	1	74.3	72.2	-2.1	77.6	75.8	-1.8	73.4	71.2	-2.2	77.9	76.0	-1.9
	2	70.1	71.1	+1.0	71.4	72.3	+.9	63.3	64.5	+1.2	74.3	75.2	+.9
	3	72.7	71.2	-1.5	75.2	73.7	-1.5	75.8	74.4	-1.4	76.8	75.5	-1.3
Sun-cured	1	78.3	82.3	+4.0	84.5	87.4	+2.9	63.9	70.6	+6.7	82.6	85.8	+3.2
	2	78.5	84.1	+5.6	83.1	87.5	+4.4	68.5	76.7	+8.2	82.1	86.8	+4.7
Artificially dried at 250° F	1	75.4	80.4	+5.0	82.6	86.1	+3.5	73.2	66.5	-6.7	83.5	86.9	+3.4
	2	77.5	84.2	+6.7	82.1	87.5	+5.4	86.0	80.1	-5.9	83.0	88.1	+5.1
	4	75.3	83.2	+7.9	82.6	88.2	+5.6	79.3	69.4	-9.9	83.2	88.6	+5.4
Artificially dried at 300° F	1	75.4	69.2	-6.2	82.8	78.3	-4.5	67.4	74.0	+6.6	82.2	77.7	-4.5
	2	76.2	72.3	-3.9	82.4	79.4	-3.0	73.0	76.9	+3.9	82.2	79.3	-2.9
	4	74.8	74.0	-.8	80.6	79.7	-.9	81.2	81.9	-.7	81.6	81.5	-.1
Artificially dried at 350° F	1	76.0	76.1	+.1	85.3	85.0	-.3	70.0	70.5	+.5	85.2	85.0	-.2
	2	75.4	78.3	+2.9	84.0	85.8	+1.8	81.2	78.7	-2.5	82.7	84.8	+2.1
	4	74.8	77.8	+3.0	84.2	86.0	+1.8	82.4	80.5	-1.9	83.5	84.9	+1.4
Artificially dried at 400° F	1	58.1	64.3	+6.2	81.3	84.1	+2.8	79.6	76.0	-3.6	76.8	80.2	+3.4
	2	59.6	66.7	+7.1	80.2	83.7	+3.5	86.4	83.5	-2.9	75.7	79.9	+4.2
	4	59.7	66.5	+6.8	81.1	84.3	+3.2	87.9	85.4	-2.5	76.4	80.4	+4.0
Average percent variation of modified from standard method		5.9 ±0.6			3.4 ±0.3			5.4 ±.7			3.5 ±0.3		
Standard deviation		3.72± .43			1.82± .21			3.94±.46			2.02± .23		
Ratio of average percent variation to its probable error		9.7			11.4			7.7			11.7		

Since the modified method of calculating apparent digestibility is dependent upon the passage of the iron through the digestive tract without absorption therefrom or additions thereto, the iron balance should provide a measure of the accuracy of the procedure. As shown in table 2, this balance varied from a retention for the 14-day period of over 20 percent of the iron ingested to an iron content of the feces of over 40 percent in excess of that ingested. In 6 cases less iron was excreted than ingested and in 11 cases more iron was excreted than ingested. These results indicate that in the case of ruminants there is not a uniform passage of the naturally occurring iron in the feed through the digestive system.

TABLE 2.—*Iron-balance data for sheep when fed green, sun-cured, and artificially dried grass*

Treatment of grass	Sheep no.	Iron ingested	Iron voided	Iron balance	Iron balance as retention of ingested iron
		Grams	*Grams*	*Grams*	*Percent*
Green	1	20.6	19.0	+1.6	7.8
	2	21.7	22.5	−.8	3.7
	3	17.1	16.1	+1.0	5.8
Sun-cured	1	18.0	22.0	−4.0	22.2
	2	18.0	24.3	−6.3	35.0
Artificially dried at 250° F	1	11.6	14.5	−2.9	25.0
	2	11.6	16.5	−4.9	42.2
	4	11.6	17.1	−5.5	47.4
Artificially dried at 300° F	1	17.9	14.3	+3.6	20.1
	2	17.9	15.4	+2.5	14.0
	4	17.9	17.2	+.7	3.9
Artificially dried at 350° F	1	11.7	11.5	+.2	1.7
	2	12.8	14.5	−1.7	13.3
	4	12.8	14.5	−1.7	13.3
Artificially dried at 400° F	1	13.5	15.9	−2.4	17.8
	2	14.8	18.0	−3.2	21.6
	4	14.8	17.8	−3.0	20.3

SUMMARY

A modified method of determining apparent digestibility was compared with the standard method in six digestion experiments with 17 sheep. The results indicate that the variations of the modified from the standard method were statistically significant.

The variations obtained in iron balances and apparent digestibility by the modified method in these experiments indicate that the use of this method is not justified in the case of ruminants.

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EFFECT OF PREVIOUS COLD STORAGE ON THE RESPIRATION OF VEGETABLES AT HIGHER TEMPERATURES [1]

By C. O. APPLEMAN, *plant physiologist*, and C. L. SMITH, formerly *assistant plant physiologist, Maryland Agricultural Experiment Station*

INTRODUCTION

It is common practice to hold fresh vegetables at low temperatures in order to retard the processes responsible for the deterioration and spoilage of the product. Much experimental work has been done to determine the storage conditions most suitable for different vegetables, but less attention has been given to the effect of the previous storage temperature on the physiology and keeping qualities of particular vegetables at market temperatures.

The high initial respiration rate previously found in potatoes (9) [2] following transfer from cold storage to higher temperatures is no doubt an important factor in the keeping qualities of such potatoes at market temperature, unless adequate ventilation is provided during this critical period. The effect of this respiratory response after a period of low-temperature storage would also apply to potatoes at the higher temperatures of late common storage if the temperature during the winter months had fallen below 5° C. for a period of 3 weeks or more. It is of both practical and scientific importance to know whether a high respiration rate for a time after cold storage is a common response of fleshy vegetables. This paper reports the results of experiments made to determine the respiratory responses at 22° of the following vegetables after various periods in storage at 2.5°: Potatoes (*Solanum tuberosum* L.), sweetpotatoes (*Ipomoea batatas* (L.) Lam.), beets (*Beta vulgaris* L.), turnips (*Brassica rapa* L.), parsnips (*Pastinaca sativa* L.), carrots (*Daucus carota* L.), and onions (*Allium cepa* L.). Dahlia roots were also included in the experiments. Besides the respiratory responses after cold storage the drift of the respiratory intensity with the age of the various vegetables was studied. Consideration was also given to the relation of the carbohydrates in the different vegetables to their respiratory intensity under different conditions, especially after cold storage.

REVIEW OF LITERATURE

As early as 1882 Müller-Thurgau (*12*) observed that potatoes which had been kept at 0° C. and then brought to 20° liberated more carbon dioxide than potatoes which had not been exposed to low tempera-

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ture. The evolution of carbon dioxide increased rapidly at first until the highest point was reached, and then sank gradually until it reached that of potatoes not previously kept at low temperature. It appears that Müller-Thurgau was concerned primarily with the effect on respiration of the sugar that accumulates in potatoes at low temperature rather than with the "change of temperature" effect.

Palladine (*16*) 17 years later exposed similar lots of tips of etiolated bean seedlings to medium, low, and high temperatures and then determined the evolution of carbon dioxide in each lot at a temperature of 18° to 22° C. The respiratory rate was stimulated by transferring the tips either from a low to a higher temperature or from a high to a lower temperature. He concluded that there is a stimulating effect on respiration caused by temperature changes, aside from the effect produced by the altered temperature.

In 1915 Appleman (*1*), using different varieties, verified Müller's earlier observation on potatoes and showed that McCormick potatoes which had been stored for 20 days at 3° C. and then brought to room temperature liberated in a unit of time 2.8 times as much CO_2 as a similar lot of potatoes that had been stored for the same length of time at room temperature. Kimbrough (*9*), working in the same laboratory, extended this study of the respiratory responses in potatoes with special reference to their storage and transportation. Potatoes were stored for different periods at constant low temperatures ranging from 0° to 10°, and the respiratory intensity in the potatoes after storage was determined at constant temperatures ranging from 10° to 30°. The practical implication of this work was to discover a temperature that would be suitable for potato storage and at the same time would reduce to a minimum the intensity and duration of the initial high respiration when the tubers were exposed to the higher temperatures.

Hasselbring and Hawkins (*7*) kept cured sweetpotatoes at a temperature of 6° to 9° C. from November 8 to December 9 and then measured the respiratory intensity of these roots at 30°. They found that the initial respiration of these chilled roots was much higher than that of roots which had not been subjected to the low temperature but, toward the end of the experiment, the respiration rates sank rapidly. Magness and Ballard (*10*) found no immediate acceleration of respiration in Bartlett pears after cold storage over fruit not held in cold storage other than the increase which would be accounted for by the slightly riper condition of the cold-storage fruit.

Magness and Burroughs (*11*) removed Winesap and Baldwin apples from storage temperatures to 18.2° C. and noted no differences that could be ascribed to the stimulation by low temperature in the rate of carbon dioxide evolution between samples from different storage temperatures. Burroughs (*4*) concluded that some stimulation of respiration occurred in the Wagener apples when they were held for 2 weeks at 0° C. and then removed to 18.5°, especially in the case of immaturely picked fruit. His data show a considerable variation in different experiments, but in general the respiration curves do not indicate the initial high respiration followed by a gradual fall to a lower level that is so characteristic of potatoes.

More recently Blackman and Parija (*3*) noted a special disturbance of initial rates of respiration in apples immediately after they were

heated from 2.5° to 22° C. The rising respiration did not simply mount up to the value for 22°, but in many cases clearly overshot that value and then fell back to it. Blackman and Parija speak of this as a minor feature that attracted attention in their study of respiration in apples.

Olney (*14*) determined the respiration rates in bananas at 20° C. He obtained a higher initial rate of respiration in refrigerated fruit than in fruit that had not been subjected to low temperature.

Hopkins (*8*) transferred potatoes from a cellar storage with an average temperature of about 10° C. to temperatures of 0° and 4.4° and measured the respiration rates at these low temperatures. The respiration rate at 0° passed through a maximum and then again approached its original value. Palladine also found a stimulation of respiration in etiolated bean seedlings immediately after they were transferred from a higher to a lower temperature. In all of the other papers here cited the initial rise in respiration rate was observed when the plant organs were transferred from a lower to a higher temperature.

EXPERIMENTAL PROCEDURE AND METHODS

GENERAL PROCEDURE

The parsnips, carrots, turnips, and beets used in these experiments were grown on a nearby commercial truck farm in fertile Sassafras loam soil. The vegetables were brought to the laboratory within an hour after harvesting and prepared immediately for the experiments. The tops were cut off one-fourth inch above the crown and all of the roots except the parsnips were washed thoroughly in running tap water. They were wiped as dry as possible with towels and then spread out on the laboratory table to dry for 2 hours before the lots were selected and weighed. The parsnips were brushed clean with a soft brush. The source and preparation of the other vegetables are described in connection with the experiments on these vegetables.

As a rule the respiration measurements at 22° C. were begun on the fresh vegetables the day they were harvested. At the same time other lots were stored at 2.5° for various periods and then transferred to 22° for respiration tests. At the beginning of all respiration measurements comparable lots were sampled for moisture and sugar determinations. The lots used for respiration were also sampled for moisture and sugar analysis at the conclusion of the respiration tests.

LOW-TEMPERATURE STORAGE

The vegetables were stored in a well-insulated box provided with a refrigeration unit which maintained in the box an average temperature of about 2.5° C. Since the fluctuations of temperature in the box were fairly regular over short intervals of time, the temperature of the vegetables in most cases was found to be fairly constant at 2.5° because of the lag in their response to small and regular fluctuations in the external temperature. The temperature of some of the small vegetables may have varied 1° to 2° at times during the storage period. Unless otherwise noted the various vegetables were placed in large moisture dishes with the lids so adjusted as to allow sufficient ventilation but to prevent excessive loss of water by evaporation during storage.

METHOD FOR MEASURING RESPIRATION

In view of the status of our knowledge of the respiratory process, it would seem that there is no entirely satisfactory method of experimentally measuring respiratory intensity in plants if we interpret this in terms of free energy. The method most commonly employed is to measure the amount of carbon dioxide liberated from a unit of plant tissue for a unit of time. There is no implication as to the metabolic source of the carbon dioxide, but it is assumed that it is the end product of a sequence of reactions in which energy is released. Although the amount of CO_2 liberated may in some cases be only an approximate measure of the total respiratory intensity, it is probably the most satisfactory measure for use in the present study. Some workers have determined the consumption of oxygen as a measure of the respiratory intensity, but since it is not definitely known just how and to what extent atmospheric oxygen enters into the total respiratory process, this method seems no more reliable than that of measuring the amount of CO_2 liberated. It is definitely known that under some circumstances oxygen may be consumed in metabolism other than respiration. Then, too, energy released by any anaerobic phase of the total respiratory process which liberates CO_2 would not necessarily be indicated by the oxygen consumption.

The method employed to measure the amount of carbon dioxide expired was similar to that described by Gore (5). The method and the apparatus used in this laboratory have been described in detail by Kimbrough (9).

In order to insure the removal of all carbon dioxide from the system and also to allow the vegetables to warm up to the temperature of the respiration chamber, carbon dioxide-free air was drawn over the material for 3 to 4 hours before the respiration measurements were begun. The expired carbon dioxide was measured at the end of each 24-hour period. All of the respiration determinations were made at a constant temperature of 22° C.

BASIS FOR CALCULATING RESPIRATION DATA

Having decided to use either the amount of carbon dioxide liberated or the amount of oxygen consumed as an approximate measure of the respiratory intensity in the living cells of a plant organ, one is still confronted with an important practical question. What basis shall be adopted for the calculation of experimental results? Shall a unit of fresh weight or a unit of dry weight be used? Because of the varying water content in plant tissues, a unit of dry weight would seem at first to be the most appropriate basis for the calculations, but this method does not take into account the possible influence of the water content on the respiratory intensity of the tissue.

Palladine (15) has proposed a method of calculation based upon the actual amount of protoplasm in the tissue. This method also leaves out of account the influence of other components of the tissue on the respiratory intensity, especially the water and carbohydrates. The variable amounts of such materials as cellulose and lignin in different plant tissues is probably the greatest source of error in comparing the respiratory intensity of different tissues and organs. Since we do not know the relative importance of the different com-

ponents of plant organs in determining their respiratory intensity as measured by the CO_2 liberated, it was thought best to use a unit fresh weight of the respiring system as the basis for calculating the experimental results of the present study.

<div align="center">ANALYTICAL METHODS</div>

Sampling.—The vegetables were split longitudinally and either one-quarter or one-half of each was used in the composite sample. In some of the early experiments with parsnips the roots were ground in a small meat chopper and then in a mortar. In all of the other experiments the vegetables were ground to a fine pulp in a Nixtamal mill. The pulp was kept thoroughly mixed while the samples were being removed for analysis.

Moisture.—From 3 to 6 g of pulp were weighed in a tared watch glass and dried to constant weight in a slow current of dry air at 78° to 80° C. and at a pressure of 28 to 30 inches of mercury.

Sugars.—The amount of pulp used for the sugar determinations varied from 20 to 50 g, depending on the sugar content of the different vegetables. The samples of pulp were weighed into counterpoised 200-cc Kohlrausch flasks and covered immediately with 75 or 100 cc of boiling 95-percent alcohol. The flasks were then placed on a steam bath, and when the contents began to boil the alcohol was reduced to 70 percent by the addition of the calculated amount of hot water and the boiling continued for 30 minutes to extract the sugars. After cooling to 20° C. the flasks were filled to the mark with 95-percent alcohol and set aside until the sugar determinations could be made.

When the sugars were determined the extracts were filtered, and 125 to 150 cc of the filtrate was evaporated on a steam bath until all of the alcohol was removed. Water was added in small amounts to keep the volume of the solution at about 40 cc. The aqueous extracts were then transferred to 200-cc volumetric flasks and cleared with neutral lead acetate. The excess of lead was removed with anhydrous sodium carbonate. The flasks were then filled to the mark and suitable aliquots of the filtered solutions were used for the sugar determinations which were made by the method of Munson and Walker (*13*).

In making the final calculations no correction was made for the volume occupied by the undissolved solids of the pulp in the volumetric flasks. Hasselbring (*6*) made the correction for the volume occupied by 25 g of carrot pulp in 250 cc of solution and found that it changed the maximum percentage of sugar only slightly in the second decimal. In the present analysis the slight error would be constant throughout and it would not affect the comparative value of the results.

The percentages of total and reducing sugars on wet-weight basis at time of sampling were calculated as percentages of the wet weight of the vegetables at the time of harvest. These latter percentages are recorded in the tables as percentages of original wet weight and they serve to check apparent changes in percentages of sugar due to loss of water and carbon dioxide from the vegetables during storage.

RESULTS

POTATOES

EXPERIMENT 1

Willaman and Brown (*18*) concluded that the temporary flush of CO_2 output from apple twigs when they were raised from a lower to a higher temperature was caused by the lower solubility of the CO_2 at the higher temperature. They further concluded that in plant-respiration studies a careful distinction must be made between the production of CO_2 and its output.

Experimental data have been reported from this laboratory (*9*) that seem to prove rather conclusively that the initial high liberation of CO_2 from potatoes after a period of cold storage is due to actual respiration and not simply to the output of the excess CO_2 which was dissolved in the cold sap of the potato tissue. As further proof of the respiratory production of excess output of CO_2 by potatoes after cold storage an additional experiment is here reported.

FIGURE 1.—Respiration in potatoes, showing that the initial high liberation of CO_2 after a period of cold storage is due largely to actual respiration.

Two comparable lots of potatoes were stored at a low temperature for the same length of time and then transferred to 22° C. for respiration tests. Carbon dioxide-free air was passed over one lot while pure nitrogen was passed at the same rate over the other lot. The carbon dioxide liberated was measured at 24-hour intervals. The results are shown in figure 1. It should be noted that most of the temporary excess of carbon dioxide liberated was from the lot in air. If the excess output of carbon dioxide were due entirely to the liberation of the carbon dioxide which was dissolved in the potato sap during the cold storage, then the lot in nitrogen should show the same temporary excess output of carbon dioxide as the lot in air, but this was not the case.

It would also be expected that most of the excess CO_2 that might be dissolved in the cold sap would be liberated as the potatoes warmed up to the respiration temperature and before the collection of the CO_2 was begun. This preliminary period was extended as long as 18 hours and the tubers were held under a high vacuum during this time, but still they showed the typical respiratory response after cold storage.

EXPERIMENT 2

Earlier respiration studies on potatoes (*9*) showed that when the tubers were stored for only 3 weeks at 2.5° C. and then transferred to 22°, the maximum initial respiration rate for these temperature conditions was obtained. The present study included an additional

experiment on potatoes which was designed to test the effect of repeating the low temperature storage for periods of 3 weeks alternating with periods of 3 weeks on respiration tests at 22°. Jersey Red Skins was the variety selected for his experiment. This variety was chosen because it showed promise of a desirable late variety for Maryland and its respiratory activity had never been determined.
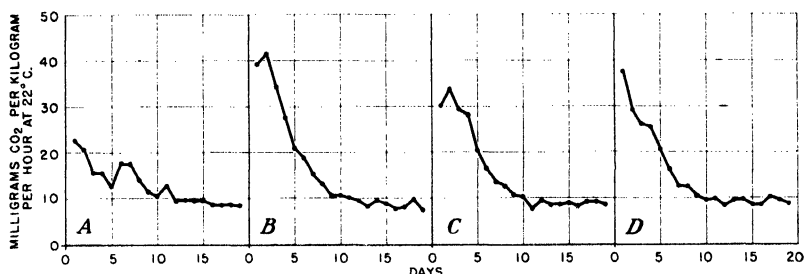
FIGURE 2.—Respiration in potatoes showing that the "change-of-temperature effect" can be reproduced at least three times in the same lot of potatoes without much change in the typical respiratory response: *A*, Respiration at 22° C., November 12 to December 6; *B*, stored at 2.5° December 6 to 29, respiration at 22° December 29 to January 19; *C*, stored at 2.5° January 19 to February 10, respiration at 22° February 10 to March 2; *D*, stored at 2.5° March 2 to 23, respiration at 22° March 23 to April 13.

The results of this experiment (fig. 2) show that the "change-of-temperature effect" can be reproduced in the same lot of potatoes at least three times over a period of 5 months without much change in the typical respiratory response.

SWEETPOTATOES

Sweetpotatoes were included in the vegetables used in this study for the purpose chiefly of comparing their respiratory responses with those of the other vegetables under similar conditions. Hasselbring and Hawkins (7) had previously conducted respiration studies with sweetpotatoes, but they were concerned primarily with the correlation between sugar content and respiration. At the same time they recognized an effect on respiration of the environmental conditions to which the sweetpotatoes had previously been subjected.

Sweetpotatoes of the Big Stem Jersey variety grown on the Maryland Experiment Station farm at College Park were dug October 25. After the roots had been brushed with a soft brush, nine lots of six roots each were selected, each lot averaging about 1,450 g in weight. Respiration measurements on one lot were begun the same day and continued until November 11. A similar lot was sampled for moisture and sugar analysis. Four lots were stored in open pans at 2.5° C. and three lots were stored at 29.3° to allow the curing processes to take place. The average of the daily mean temperatures for 22 days prior to the date when the sweetpotatoes were harvested was 13.7°.

The periods of storage at the different temperatures, together with respiration and analytical results, are shown in table 1, and in figure 3. The respiration data on the sweetpotatoes that were stored immediately after harvest at 2.5° C. for 24 days are not included. Although these sweetpotatoes were sound at the end of the cold-storage period, soft rot appeared in three of the roots during the respiration tests. All of the sweetpotatoes that had been allowed to cure before they

were placed in cold storage remained sound during the respiration measurements. The rate of respiration, which was high in the freshly harvested sweetpotatoes (fig. 3, *A, a*), declined rapidly at
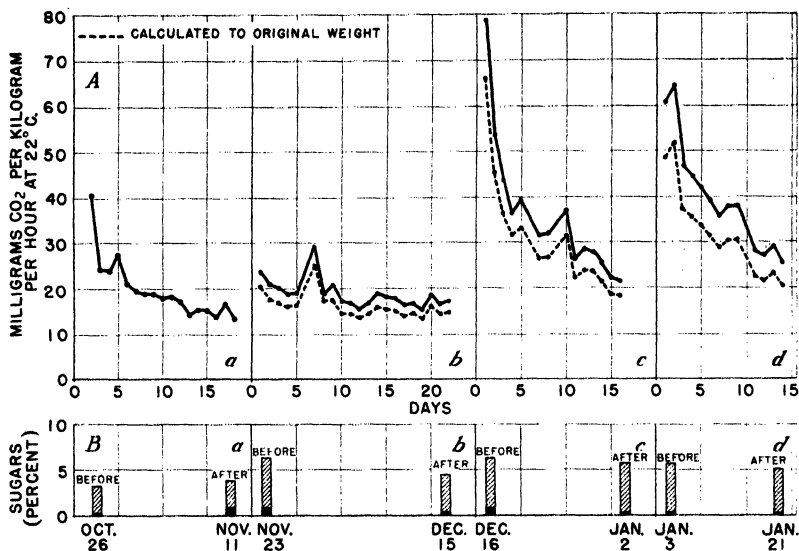
FIGURE 3.—*A*, Respiration in sweetpotatoes before and after storage at temperatures of 29.3° and 2.5° C.: *a*, Lot 1, at time of harvest; *b*, lot 2, after storage at 29.3° for 28 days; *c*, lot 3, after storage at 29.3° for 28 days, then at 2.5° for 23 days; *d*, lot 2, after the first respiration test stored again at 2.5° for 23 days. *B*, Sugar content of the different lots before and after the respiration tests. The height of the columns indicates total sugars and the shaded portions reducing sugar.

first and then more slowly for about 12 days, when it began to fluctuate around a fairly constant average for the remainder of the 17-day respiration period.

TABLE 1.—*Moisture and sugars in freshly harvested and in cured sweetpotatoes before and after storage at 2.5° C.*

FRESHLY HARVESTED, BEFORE AND AFTER STORAGE

Storage period (days)	Moisture	Total sugars			Reducing sugars		
		Wet weight	Original wet weight	Dry weight	Wet weight	Original wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	77.02	3.26	3.26	14.18	0.33	0.33	1.45
24	74.82	5.92	5.16	23.52	.61	.53	2.44

CURED, BEFORE AND AFTER STORAGE

| 0 | 75.68 | 6.23 | 5.24 | 25.62 | 0.81 | 0.68 | 3.29 |
| 23 | 74.64 | 6.32 | 5.31 | 24.95 | .81 | .68 | 3.21 |

When the cured sweetpotatoes were stored at 2.5° C. and then transferred to 22° their initial respiration rate was nearly double that of the freshly harvested roots (fig. 3, *A, c*). The respiration rate declined rapidly for about 5 days and then irregularly and much more

slowly until it nearly reached, in 15 days, the rate in the cured sweet-potatoes before they were placed in cold storage.   The sweetpotatoes that had been used for respiration tests after a period of curing (fig. 3, *A*, *d*) were also stored at 2.5° at the end of the respiration period.   After 23 days at 2.5° the drift of the respiration curve at 22° was very similar to the one just described, although the initial rate was not as high.

The freshly harvested sweetpotatoes contained much less total and reducing sugars than the cured roots, but the initial respiration rate was much higher in the fresh uncured sweetpotatoes.   During the respiration period of the freshly harvested roots the total sugars increased from 14.18 to 17.43 percent dry weight and the reducing sugars from 1.45 to 4.70 per-cent, but the rate of respiration declined to less than one-half of the initial rate.   The percentage of both total and reducing sugars in the cured sweetpota-toes did not show any signifi-cant increase during 23 days' storage at 2.5° C. but the initial respiration rate in the roots after the cold-storage period was 3.5 times greater than the rate in cured roots before cold storage. The data from these experi-ments do not indicate a general correlation between either the total or reducing sugar content of the sweetpotato and its re-spiratory activity.   The exper-iments of Hasselbring and Hawkins (7) likewise showed no correlation between total sugar content and respiration, but they claimed that their experi-ments indicated a correlation

FIGURE 4.—*A*, Respiration in Detroit Dark Red and Crosby Egyptian beets before and after storage at 2.5° C.: *a*, Lot 1, at time of harvest; *b*, lot 2, after 26 days' storage; *c*, lot 2, after a second period, this time of 32 days, in cold storage.   *B*, Sugar content of the different lots before and after the respiration tests. The height of the columns indicates total sugars and the shaded portions reducing sugars.

between reducing sugar content and respiration, although they admitted that such a correlation might be obscured by other factors.

<div align="center">BEETS</div>

Two varieties of beets, Crosby Egyptian and Detroit Dark Red, were harvested October 23, and 4 lots of 10 beets each were selected from each variety.   The average weight per lot was about 950 g for the Detroit Dark Red beets and about 830 g for the Crosby Egyptian. The experiments on the former variety were begun the day they were dug, and on the latter variety 2 days later.

At the end of the first cold-storage period, respiration measurements were continued for 8 days.   The beets were then subjected to a second period of cold storage, and respiration tests made again for 4 days. The results of this experiment together with the storage periods are shown in table 2 and figure 4.   The percentages of sugar in the two

varieties were so nearly the same that only those for the Detroit Dark
Red are plotted.

TABLE 2.—*Moisture and sugars in Detroit Dark Red and Crosby Egyptian beets at
time of harvest and after storage at 2.5° C.*

DETROIT DARK RED

Storage at 2.5° C. (days)	Moisture	Total sugars			Reducing sugars		
		Wet weight	Original wet weight	Dry weight	Wet weight	Original wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	88.77	5.56	5.56	49.54	0.08	0.08	0.68
28	88.02	5.56	5.37	46.51	.10	.09	.82

CROSBY EGYPTIAN

Storage at 2.5° C. (days)	Moisture	Total sugars			Reducing sugars		
0	86.85	6.65	6.65	50.64	0.09	0.09	0.75
26	85.46	6.68	5.69	45.89	.11	.09	.81

The general drift of the respiration curve was the same in the two
varieties, but the rate in the Crosby Egyptian beets was consistently
higher at all times. The initial rate of respiration in the beets after
the first period of cold storage was less than that in the freshly har-
vested beets, but the decline in rate with time was about the same.
The initial rate of respiration after the second storage period at low
temperature was considerably lower than the initial rate after the
first period of cold storage, but it was nearly double the rate in the
beets before the second storage, thus showing some effect on respira-
tion rate of the sudden transfer of the beets from cold to warm tem-
perature. No correlation of respiration rate with the sugar content
could be detected in the beets. The much higher respiration in the
Crosby Egyptian beets after the second period of cold was not due to a
difference in sugar content, for the two varieties differed in this respect
by only 0.05 percent.

TURNIPS

The turnips used for the experiments were the Purple Top White
Globe. variety, and were harvested October 23. Four lots were
selected, 2 lots of 10 roots each and 2 lots of 8 roots each. Respira-
tion measurements were started on one lot and another lot was
sampled for analysis. The remaining two lots were stored at 2.5° C.
for 28 days, after which one lot was sampled for analysis and the other
was used for respiration tests for a period of 8 days. This lot was
stored a second time at 2.5° for 32 days and then respiration measure-
ments were made for 5 days. The results are given in table 3 and
figure 5. The average of the mean daily temperature for a period of
3 weeks prior to the harvesting date was 14.3°.

The respiration rate in the freshly harvested turnips decreased
rapidly during the first 2 days and then much more slowly but con-
tinuously until the end of the respiration measurements. The
initial rate in the turnips that had been in cold storage was consider-
ably less than the initial rate in the freshly harvested turnips, but
after a temporary rise the decline in rate was about the same.

TABLE 3.—*Moisture and sugars in turnips at time of harvest and after storage at 2.5° C.*

Storage period (days)	Moisture	Total sugars			Reducing sugars		
		Wet weight	Original wet weight	Dry weight	Wet weight	Original wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	91.93	4.14	4.14	51.34	3.67	3.67	45.58
28	91.87	4.17	4.05	51.30	3.77	3.66	46.49

The respiration curve for the turnips after the second period of storage at low temperature was in general similar to that for the roots after the first cold-storage period. The initial rate was much higher than that in the roots just before the second cold storage. This would seem to indicate some change-of-temperature effect on the respiration in turnips.

In the fresh turnips the percentage of reducing sugars was much higher than the percentage of sucrose, which was relatively low in these vegetables. During storage of the roots at the low temperature there was a slight increase in reducing sugars, but there was no appreciable change in the total sugar during the 28 days of storage. It is apparent from the results of this experiment that there is no direct relationship between sugar content and intensity of respiration in turnips.

FIGURE 5.—*A*, Respiration in turnips before and after storage at 2.5° C.: *a*, Lot 1, at time of harvest; *b*, lot 2, after 28 days' storage; *c*, lot 2, after a second period, this time of 32 days, in cold storage. *B*, Sugar content of the different lots before and after the respiration tests. The height of the columns indicates total sugars and the shaded portions reducing sugars.

PARSNIPS

Parsnips of the Hollow Crown variety were harvested November 11 and brought immediately to the laboratory. The tops were cut off about one-fourth inch above the crowns and the dirt removed from the roots by brushing with a soft brush. Ten lots of 8 roots each were selected for respiration determinations and 10 lots of 5 roots each were selected for sugar and moisture determinations. The lots used for respiration averaged about 700 g each, while those for sugar analysis averaged about 500 g each. One lot was placed immediately in the respiration chamber and another lot was sampled for sugar and moisture analysis. Eight lots were stored at 2.5° C. The roots for storage were placed on soil in 10-inch earthenware pots and covered with soil to a depth of about 1 inch. The pots were set in shallow pans in which was kept a little water to keep the soil damp. Under these conditions the roots actually increased slightly in weight

during the long storage periods and the percentage of water in the roots increased as shown in table 4.

TABLE 4.—*Moisture and sugars in parsnips at time of harvest and after soil storage at 2.5° C.*

Storage period (days)	Moisture	Total sugars		Reducing sugars	
		Wet weight	Dry weight	Wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
0	79.20	4.30	20.67	0.20	1.11
21	78.75	7.00	32.91	.43	2.01
47	81.32	7.79	41.65	.40	2.14
92	83.10	7.17	42.50	.79	4.68
131	83.47	8.20	49.65	.65	3.95

Duplicate lots of roots were removed from storage for respiration tests and sugar and moisture determinations after 21, 47, 92, and 131 days in storage. The results on the soil-stored roots are shown in table 4 and in figure 6.
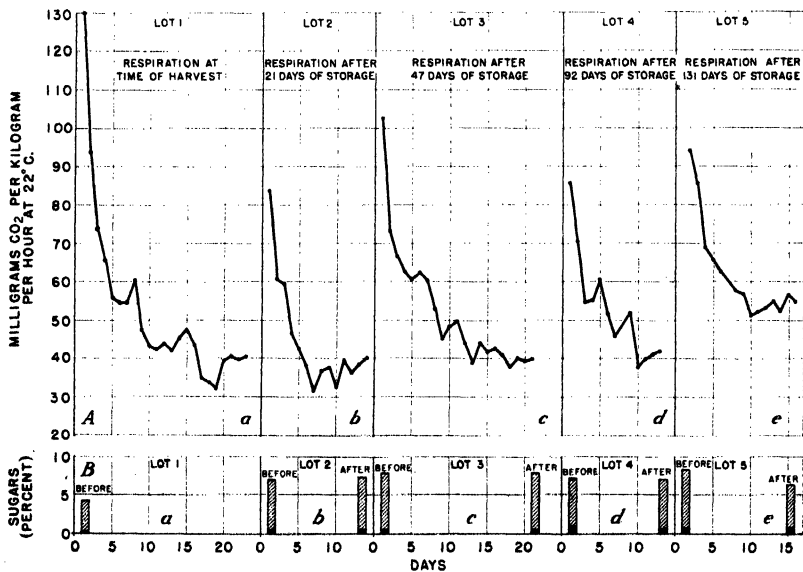
FIGURE 6.—*A*, Respiration in parsnips before and after storage in soil at 2.5° C.: *a*, Lot 1, at time of harvest; *b*, lot 2, after 21 days' storage; *c*, lot 3, after 47 days' storage; *d*, lot 4, after 92 days' storage; *e*, lot 5, after 131 days' storage. *B*, Sugar content of the different lots before and after the respiration tests. The height of the columns indicates total sugars and the shaded portions reducing sugars.

The high rate of respiration in the parsnips immediately after harvest declined rapidly and steadily for the first 5 days and then much more slowly and irregularly for the remainder of the respiration period. The respiration in these roots seemed to be approaching a fluctuating rate around a fairly constant mean. Small shoots developed on some of the parsnips on the seventeenth day, but caused no apparent increase in the respiration rate. The field temperature

previous to harvesting may have had some effect on the initial rate of respiration in the fresh parsnips, as the average of the mean daily temperature for a period of 3 weeks just prior to harvesting the roots was 6.6° C. This was much lower than the temperature at which respiration determinations were made but was higher than the storage temperature.

The general drift of the respiration rate in the parsnips after the different periods of cold storage was very similar to that in the freshly harvested roots except that the initial rate was never as high and the final rate after the long storage periods was not as low as in the fresh parsnips.

There was no direct correlation between the sugar content of the parsnips and the rate of their respiration. The initial rate in the unstored lot was higher than that in any other lot, but the percentage of both reducing sugars and total sugar was lower than in any other lot. The lot stored for 21 days had about twice as much reducing sugar and more than a third more total sugar than the original fresh lot at the beginning of the respiration period.

Several lots of parsnips were also placed in small wire baskets and stored at the low temperature. The loss of water from these air-stored lots was so great that the respiration data are of interest only in showing the effect of water content on the respiratory activity of these roots. The relationship of both moisture and sugar content to respiration is shown in table 5. In this table only the respiration rate for the second day of each test is recorded. These figures seem just as satisfactory for comparing the respiratory activity in the different lots as the data for the entire period of the respiration test. After 47 days' storage the respiration rates per unit of dry weight were nearly the same in the soil- and air-stored lots,

FIGURE 7.—Total sugar content (percentage) of parsnips when stored in air or in soil at 2.5° C.

although the soil-stored lots contained 81.32 percent moisture while the air-stored lot contained only 57.45 percent. A further reduction of the percentage of moisture in the air-stored parsnips resulted in a corresponding decrease in respiration until it reached a very low value when the moisture content was reduced to 31.65 percent. After 21 days' storage the respiration rate of the air-stored parsnips was nearly twice as great as that of the soil-stored lot. This may or may not have been due to the fact that the sugar content after 21 days' storage was much higher in the air-stored lot and the water content was still optimum for respiratory activity.

It is a significant fact demonstrated by repeated experiments that the accumulation of sugar in parsnips at low temperature is much more rapid when the parsnips are freely exposed to air than when they are buried in the soil. (fig. 7.).

TABLE 5.—*Relation between respiration rate of parsnips and their moisture and sugar content after varying periods of storage at 2.5° C.*

[All percentages calculated to dry basis]

Storage period (days)	CO₂ per gram dry weight per hour		Moisture		Total sugars		Reducing sugars	
	Stored in soil	Stored in air	Stored in soil	Stored in air	Stored in soil	Stored in air	Stored in soil	Stored in air
	Mgm	*Mgm*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
21	6. 83	12. 75	78. 75	74. 75	32. 91	51. 71	2. 01	7. 10
47	9. 41	8. 89	81. 32	57. 45	41. 65	48. 35	2. 14	5. 88
92	9. 98	3. 82	83. 10	39. 88	42. 50	46. 65	4. 68	2. 75
131	13. 63	. 74	83. 47	31. 65	49. 65	44. 05	3. 95	2. 40
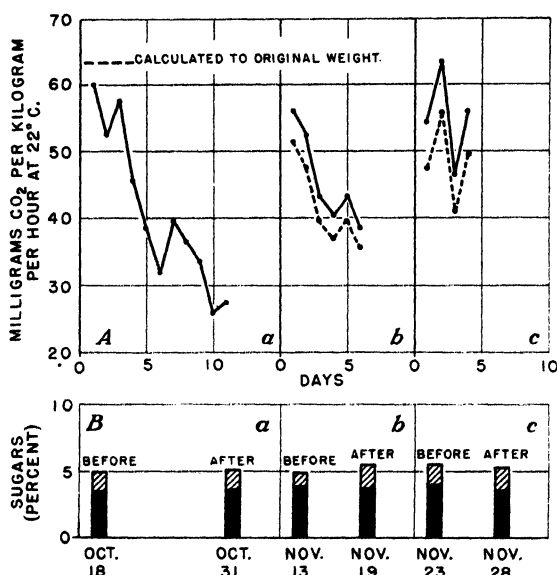
FIGURE 8.—*A*, Respiration in carrots used in experiment 1 before and after storage at 2.5° C.: *a*, Lot 1, at time of harvest; *b*, lot 2, after 26 days' storage; *c*, lot 3, after 36 days' storage. *B*, Sugar content of the different lots before and after the respiration tests. The height of the columns indicates total sugars and the shaded portions reducing sugars.

CARROTS

EXPERIMENT 1

Danver Half-Long or closely related varieties were used for all of the experiments with carrots. The carrots for experiment 1 were harvested October 16, and stored in crates in a cool cellar until October 17, when lots of 15 carrots each were selected. Two lots were used for immediate respiration tests and one lot was sampled for sugar and moisture determinations. The remaining lots were stored at 2.5° C. At the end of 26 days and 36 days respectively, triplicate lots were removed for respiration tests and for moisture and sugar determinations. The results are shown in table 6 and in figure 8. The averages of the respiration rates of duplicate, lots are plotted.

TABLE 6.—*Moisture and sugars in freshly harvested carrots and after storage at 2.5° C.*

Storage period C (days)	Moisture	Total sugars			Reducing sugars		
		Wet weight	Original wet weight	Dry weight	Wet weight	Original wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	88. 60	5. 07	5. 07	44. 55	3. 48	3. 48	30. 62
26	88. 95	5. 02	4. 53	45. 51	4. 02	3. 62	36. 48
36	87. 85	5. 58	4. 93	46. 06	4. 11	3. 63	33. 92

<div align="center">EXPERIMENT 2</div>

Repeated respiration experiments with fresh carrots all showed a rapid fall in respiration rate with age of the roots. The initial respiration rate after a period of cold storage was never as high as the rate at time of harvest. This experiment was planned especially to decide whether the respiratory response in carrots after cold storage is a change-of-temperature effect or whether it merely represents the natural drift of the respiration rate due to senescence of the roots at the respiration temperature.

The carrots were harvested in the morning of November 12 and brought immediately to the laboratory and prepared for the experiment. Five lots of 10 carrots each were selected and each lot was treated as indicated in table 7.

TABLE 7.—*General plan of experiment 2, with carrots, and the loss in weight of each lot during each experimental period*

Lot no.	Period A, 2 weeks	Loss in weight	Period B, 3 weeks	Loss in weight	Period C, 2 weeks	Loss in weight
		Percent		*Percent*		*Percent*
1	Stored at 22° C	3.03	Stored at 2.5° C	1.75	Respiration 22° C	1.2
2	____do	2.76	Stored at 22° C		____do	1.8
3	____do	2.81	____do	4.7	____do	1.4
4	Respiration 22° C	3.12	Stored at 2.5° C	3.1	____do	1.4
5	Stored at 2.5° C	3.20	Respiration 22° C	2.3		

During period A moist air was passed through the chambers containing lots 1 and 2 at the same rate as for the respiration tests. Moist carbon dioxide-free air was passed through the chamber containing lot 3 in order to make the storage conditions of this lot exactly comparable to those of lot 4, on which respiration tests were being made during this period. Lots 1 and 2 were intended to check the effect of passing carbon dioxide-free air over lots 3 and 4 during period A. During period B all of the stored lots were placed in moist chambers with the covers slightly raised to allow ventilation.

The results of this experiment are shown in figure 9. The final respiration results for lots 1 and 2 were not plotted as they did not vary from those for lots 3 and 4 more than would be expected in different lots of carrots.

FIGURE 9.—Respiration in various lots of carrots, used in experiment 2 and shown in table 7, after storage at various temperatures for different periods.

It seems quite evident from this experiment that the apparent change-of-temperature effect in carrots is simply the drift of respiration rate with senescence of the roots at the respiration temperature. The period of cold storage simply postponed the inevitable falling of the respiration rate with age.

EXPERIMENT 3

Another experiment was conducted with carrots of a crop grown the following year (1930) in order to test further the results and conclusions of experiment 2. The seed for this crop was sown in June of the very dry season of 1930. When the carrots were dug on October 21 the soil around the roots was almost a mass of dust, but nevertheless the moisture content of the carrots was 84.6 percent. Carrots from crops grown during the three previous seasons had had a moisture
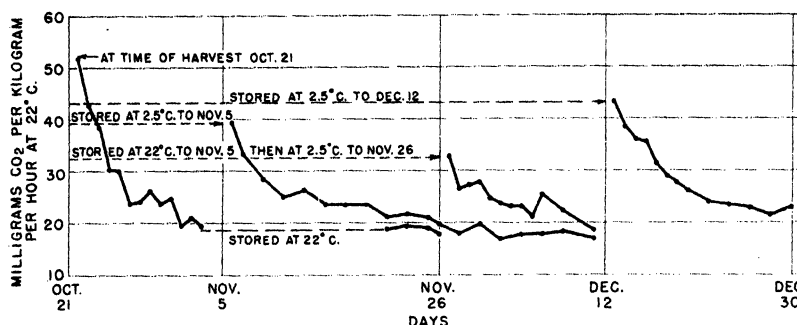
FIGURE 10.—Respiration in various lots of carrots used in experiment 3, after storage at temperatures of 22° and 2.5° C. for different periods to demonstrate the effect of senescence upon respiration.

content of 88.0, 87.3, and 88.6 percent, respectively. On the day the carrots were harvested five lots of eight roots each were selected for the experiment. The lots ranged in weight from 814.5 to 870.5 g, the average weight being 848 g. The respiration results as well as the storage treatments are shown in figure 10. The lot stored at 22° C. was placed in the respiration chamber with two glass tubes extended through the stopper to furnish ventilation. The lots stored at 2.5° were placed in moist chambers with the lids raised slightly. Under these conditions the roots lost little weight and remained sound for the period of the experiment. During the period of respiration measurements at 22° there was always more or less shoot growth but this growth did not appear to affect the respiration rate of the roots as measured by the carbon dioxide expired. Frequent removal of the shoots did not materially alter the respiration rate as compared with that of a lot from which the shoots were not removed.
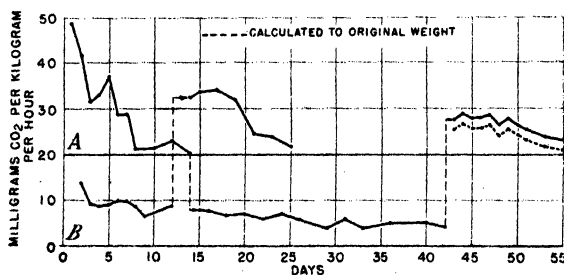
FIGURE 11.—Respiration in various lots of carrots used in experiment 4, stored at alternate temperatures of 22° (*A*) and 2.5° C. (*B*) for various periods.

EXPERIMENT 4

In experiment 4 the carrots were exposed to alternate temperatures of 2.5° and 22° C., but this experiment differed from previous ones in that respiration measurements were made at both the high and low temperature and were continued throughout the experiment. The roots therefore were exposed continuously to the experimental con-

ditions of the respiration tests. The experimental procedure and the respiration results are given in figure 11.

<div align="center">EXPERIMENT 5</div>

Since carrots are commonly shipped and handled with the tops attached, respiration tests were made on roots and tops attached and on roots and tops separately. The experiment also served to test the effect of the wound stimulus when the tops are removed from the roots.

The carrots for this experiment were harvested November 30 and brought immediately to the laboratory. Lots of 10 roots each were selected. The tops were removed from one lot and the roots were placed at once in the respiration chamber. At the same time respiration tests were begun on another lot with tops attached. After 3 days the tops were removed from this lot and respiration tests continued on the roots and tops separately. The results are shown in table 8.

TABLE 8.—*Respiration in carrots with tops attached and in roots and tops separately*

Part and treatment	CO₂ per hour per kilo for 24-hour period indicated									
	1	2	3	4	5	6	7	8	9	10
	Mgm	*Mgm*	*Mgm*	*Mgm*	*Mgm*	*Mgm*	*Mgm*	*Mgm*	*Mgm*	*Mgm*
Roots	76.0	81.5	61.5	52.0	54.1	48.5	49.7	44.7	37.3	41.9
Roots and tops attached	103.4	125.8	104.8							
Roots after removal of tops				63.0	57.6	52.4	50.9	47.3	42.4	39.7
Tops				268.6	311.2	313.3				
Calculated values for root and top attached				107.1	110.9	108.3				

The respiration rate for roots and tops attached was more than double that of an equal weight of roots alone. The respiration rate of the detached tops was 4 to 6 times higher than that for an equal weight of roots.

Since the ratio of root weight to top weight was known, it was possible from the data on roots and tops separately to calculate the respiration rate for roots and tops attached.

A comparison of these calculated values with the actual data for respiration of roots and tops attached shows that there was only a very slight increase in respiration rate in the roots due to the wound stimulus from removal of the tops.

Platenius (17) states that the practice of storing bunched carrots even for a relatively short time cannot be recommended. He found that water moves at first from the roots into the leaves at a rapid rate, but later when the tops begin to die the water is drawn back into the roots. The high respiration in the tops (table 8) suggests the possible movement of solutes from roots to tops, as the carbohydrates in the tops would soon be exhausted by the high respiratory intensity at 22° C.

<div align="center">ONIONS</div>

On October 17, onions of a yellow variety grown in Michigan were purchased on the market. On October 18, six lots of 10 onions each were selected. The average weight per lot was about 1 kg. Respira-

tion measurements were started the same day on one lot and a second lot was sampled for analysis. The other lots were stored at 2.5° C. in paper bags, which were perforated in a few places to insure ventilation. Duplicate lots were removed from storage at the end of 23 and 49 days, respectively, for analysis and respiration determinations. The results are given in table 9 and in figure 12.

TABLE 9.—*Moisture and sugars in onions before and after storage at 2.5° C.*

Storage period (days)	Moisture	Total sugars			Reducing sugars		
		Wet weight	Original wet weight	Dry weight	Wet weight	Original wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	90.50	6.42	6.42	67.57	3.26	3.26	34.32
23	90.44	6.40	6.30	66.91	3.61	3.55	37.79
49	90.85	5.87	5.66	64.22	3.79	3.85	41.43

The respiration rates of onions were relatively low in all cases, but the initial rates in the fresh onions and in those after periods of cold storage were higher than the final rates attained for the period of the tests. The initial rates after storage were practically the same as the initial rate in fresh onions, but the final rates were lower than the final rate of the fresh onions.

The total sugar content of these onions was relatively high, being 67.5 percent of the
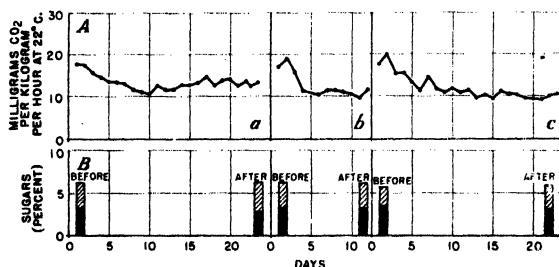
FIGURE 12.—*A*, Respiration in onions before and after storage at 2.5° C.: *a*, Lot 1, before storage; *b*, lot 2, after 23 days' storage; *c*, lot 3, after 49 days' storage. *B*, Sugar content of the different lots before and after the respiration tests. The height of the columns indicates total sugar and the shaded portions reducing sugars.

dry weight. The reducing sugars accounted for approximately one-half of the total sugars. During cold storage there was no accumulation of total sugar in the onions, but the reducing sugars seemed to increase slightly with the cold-storage period. From the data obtained there appears to be no general correlation between sugar content and respiration in onions.

DAHLIA ROOTS

Dahlia roots were included in this study because they store reserve carbohydrates in the form of inulin which upon hydrolysis gives rise to levulose. The roots are also stored for long periods.

The dahlia roots used in this experiment had been stored in a cool cellar from the time of harvest until February 29, when they were brought to the laboratory and prepared for the experiment.   The roots were cut from the stalks, washed in running tap water, wiped with towels, and allowed to dry on the laboratory table for 2½ hours.   Four lots of 11 roots each were then selected. The average weight per lot was about 760 g.   Respiration determinations were started immediately on one lot, and one lot was sampled for analysis.   The other two lots were placed in ventilated moist chambers and stored at 2.5° C. for 23 days. At the end of this period one lot was used for respiration tests and the other for analysis.   The lot used for the respiration measurements

FIGURE 13.—*A*, Respiration in dahlia roots before and after storage at 2.5° C.: *a*, lot 1, before storage; *b*, lot 2, after 23 days' storage; *c*, lot 2, after a second period of 23 days' storage at 2.5° C.   *B*, Sugar content of the different lots before and after the respiration tests.   The height of the columns indicates total sugars and the shaded portions reducing sugars.

for a period of 8 days was again stored at 2.5° for 21 days, after which respiration determinations were made again for 11 days. The results are given in table 10 and figure 13.

TABLE 10.—*Moisture and sugars in dahlia roots before and after storage at 2.5° C.*

Storage period (days)	Moisture	Total sugars			Reducing sugars		
		Wet weight	Original wet weight	Dry weight	Wet weight	Original wet weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	82.11	3.47	3.47	19.40	0.67	0.67	3.75
23	81.23	4.16	3.80	22.20	1.39	1.27	7.40

The type of respiratory response in the dahlia roots after a period of cold storage was very similar to that in potatoes and sweetpotatoes, although the rate in the latter was much higher.   Both the total and reducing sugar increased during the cold-storage period.

COMPARISON OF RESPIRATION RATES OF DIFFERENT VEGETABLES IMMEDIATELY AFTER HARVEST

A comparison of the respiration rates of the different vegetables immediately after harvest is presented in figure 14. This comparison is based upon equal weights of the fresh vegetables and also upon equal weights of dry matter. On the fresh-weight basis the vegetables ranked in the following descending order in respect to initial respiratory intensity: Parsnips, carrots, beets, turnips, sweetpotatoes, potatoes, and onions. On the dry-weight basis the vegetables ranked in the following descending order: Turnips, parsnips, carrots, beets, onions, and sweetpotatoes. The shifting in the order of the vegetables when the respiration rate is based upon wet and dry weight cannot be explained entirely by the percentage of moisture in the various vegetables. The time rate of respiration in the dahlia roots was very similar to that in potatoes.

FIGURE 14.—Comparative respiration rates of various vegetables, and of dahlia roots, immediately after harvest: *A*, Onion; *B*, sweetpotato; *C*, potato; *D*, dahlia root; *E*, turnip; *F*, parsnip; *G*, carrot; *H*, beet.

RELATION OF SUGAR CONTENT TO RESPIRATION

The vegetables that accumulated sugar at low temperature were the ones in which the respiration rates at 22° C. were most altered by previous cold storage. Müller-Thurgau (*12*) observed that the rate of respiration in potatoes when they were transferred from a low to a higher temperature gradually declined as the sugar in the tubers was reconverted into starch. He therefore concluded that the sugar which accumulated in the tubers during cold storage was responsible for the initial increase in respiratory intensity when they were transferred to a higher temperature.

The experiments here reported failed to show any direct relationship between sugar content and respiration rate in the vegetables studied. The following typical experiments afford further evidence that Müller-Thurgau was mistaken in his conclusions regarding the relation of sugar content to respiration in potatoes.

EXPERIMENT 1

Potatoes that had been at low temperature long enough to accumulate 1.5 percent of sugar were transferred to 30° C. for respiration

measurements.   The drift of respiration rate was typical, being h gh at first and then declining gradually to a much lower rate.   During the period of the most rapid decline in the respiration rate the percentage of both total and reducing sugars in the tubers was actually increasing.   It has long been known that the accumulated sugar in potatoes during cold storage is reconverted into starch when the potatoes are transferred to a warmer temperature.   Therefore it was surprising to find the sugar continuing to increase for a time at 30° (fig. 15).   However, further studies showed that in potatoes the shifting equilibrium between sugar and starch tends to attain stability with widely different percentages of sugar characteristic of different temperature ranges.   In the variety of potatoes used in this experiment the equilibrium at 30° was attained when the total sugar in the potatoes was about 2.5 percent.   Since the tubers contained only about 1.5 percent total sugar when removed from the low temperature the sugar continued to increase until equilibrium characteristic of 30° was established.   In another experiment the tubers contained 4.3 percent sugar when transferred from low temperature to 30°, so the sugar in these potatoes decreased until it was reduced

FIGURE 15.—Relation between total sugars, reducing sugars, and respiration rate of potatoes held at 30° C.

to 2.5 percent characteristic of equilibrium at 30°.   Since the carbohydrate equilibrium is attained at 20° to 25° when the total sugar content is a fraction of 1 percent the usual observation is a marked decrease in the sugar content of cold-storage potatoes, after they are removed to a warmer temperature.   It was therefore natural to infer that these carbohydrate transformations in potatoes with temperature changes were the cause of the characteristic respiratory response with the same temperature changes.

<center>EXPERIMENT 2</center>

McCormick potatoes that had been stored for a time at 29° C. were found to contain 2.05 percent total sugar.   A lot of these potatoes was used for respiration measurements.   After the respiration tests the tubers were placed at room temperature for 7 days and then stored at 0° for 12 days.   After the period of cold storage they contained 2.18 percent total sugar.   Respiration measurements were again made on a sample of this lot of tubers.   The results, shown in table 11, indicate that the initial respiration rate in the cold-storage potatoes at the time that they were moved to 29° was more than two and one-half times the initial respiration at the same temperature in the tubers after storage at 29°, although the total sugar content was practically the same in both lots of potatoes.

TABLE 11.—*Relation between respiration rate of potatoes and their sugar content after storage at 29° and 0° C.*

Previous storage temperature (° C.)	Total sugar	CO_2 per kilogram per hour at 29° C.	
		First 24 hours	Second 24 hours
	Percent	*Milligram*	*Milligram*
29	2.05	29.3	24.3
0	2.18	77.8	29.7

DISCUSSION

The respiratory intensity in the different vegetables immediately after harvest varied greatly. The time rate of respiration in potatoes, sweetpotatoes, parsnips, onions, and dahlia roots declined rapidly at first and then more slowly until it began to fluctuate around a faily constant average rate for the remainder of the respiration period. In the case of carrots, beets, and turnips, the respiration rate declined steadily until the end of the respiration period. Benoy (*2*) subjected 10 different kinds of green vegetables of edible maturity to respiration tests during the first 30 hours after they were harvested. Two of the vegetables, carrots and beets, were used in the present study, and the general trend of respiration in these vegetables immediately after harvest was much the same as that found by Benoy, except that in her experiments respiration seemed to approach a constant rate after the first 12 hours. This may have been due to the fact that her experiments lasted only 30 hours.

Since the initial rate of respiration in the freshly harvested vegetables declined during the period of the respiration tests it was difficult to decide whether the respiratory response after cold storage was due to a sudden change of temperature or whether it merely represented the natural drift of the respiration rate due to the senescence of the vegetables at the respiration temperature. Experiments especially planned to decide this point showed that the high initial respiration rate in potatoes, sweetpotatoes, and dahlia roots when they are suddenly changed from a lower to a higher temperature is a definite change-of-temperature effect.

Potatoes were subjected to 3 weeks' storage at 2.5° C. and then to 3 weeks' respiration tests at 22°. This procedure could be repeated three times on the same lot of potatoes over a period of 5 months without any significant change in the typical respiratory response after each succeeding period of cold storage. Potatoes responded only to a change from a lower to a higher temperature and not to a change from a higher to a lower temperature.

There was also a distinct change-of-temperature effect on the respiration rate of beets and turnips, but it was very much less pronounced.

The respiration rate in carrots when they were transferred from a low to a higher temperature declined rapidly and steadily. This might easily have been interpreted as a change-of-temperature effect

but it proved to be the natural drift of the respiration rate during senescence of the roots. The senescence processes were merely deferred during the periods of cold storage.

The drift of the respiration rate in parsnips after varying periods of cold storage was very similar to that in the freshly harvested roots at the same temperature, but the initial rate after cold storage was never as high as the initial rate in the fresh parsnips. The experiments with parsnips do not permit definite conclusions regarding the change-of-temperature effect on the respiration rate in these roots.

The respiration rate in the onions was low at all times and previous cold storage had little or no effect on the respiration rate at the constant higher temperature.

SUMMARY

A comparative study was made of the change-of-temperature effect on the time rate of respiration in a number of fleshy vegetables. The respiration measurements were made at 22° C. after the vegetables had been stored for varying periods at 2.5°. The drift of the time rate of respiration with age of the various vegetables was included in the study. Consideration was also given to the relation of moisture and carbohydrates in the different vegetables to their respiration intensity.

The choise of a method for measuring respiration intensity and the proper basis for calculating respiration data are discussed.

Further experimental proof of the respiratory production of the excess output of carbon dioxide by potatoes after cold storage is reported.

The effect of previous cold storage on the initial respiration rate at higher market temperatures varied with the different vegetables, being very pronounced in potatoes but not detectable in carrots. The vegetables in which the percentage of starch was relatively high and in which there was a rapid shifting of the carbohydrate equilibrium with temperature changes were the ones that showed the greatest increase in intial respiratory rate when they were transferred from a low to a higher temperature. The actual respiratory intensity in these vegetables, however, was lower than in most of the other vegetables studied.

The sugar that accumulates in starchy vegetables at low temperature is not responsible for the initial high respiration in these vegetables when they are moved to higher temperatures. In potatoes the shifting equilibrium between sugar and starch tends to attain stable positions with widely different percentages of sugar characteristic of different temperature ranges. Potato tubers accumulated sugar for a time at 30° C. as well as at temperatures just above freezing, but the sugar that accumulated in the tubers at the high temperature did not affect the respiration rate.

There was no direct correlation between the content of either total or reducing sugars and the respiration rate in the vegetables studied.

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THE pH GRADIENT EXTENDING FROM THE PHLOEM INTO THE PARENCHYMA OF THE SUGAR BEET AND ITS RELATION TO THE FEEDING BEHAVIOR OF EUTETTIX TENELLUS [1]

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INTRODUCTION

Certain preliminary studies pertaining to the chemical nature of resistance to the curly top disease indicated the possibility that differences in hydrogen-ion concentration might be involved. The pH value of the expressed juice of a number of species of plants showing varying degrees of resistance to curly top was determined with the aid of the quinhydrone electrode. The freshly extracted juice from *Chenopodium murale* L., a very resistant species, consistently showed a distinctly alkaline reaction (pH 7.3 to 8).[2] By means of the same electrode it was found that juice extracted from sugar beet leaves was always acid (pH 5.8 to 6.5).

From these observations the suggestion arose that if by subjecting *Chenopodium murale* to a high concentration of carbon dioxide the cell sap of the plant could be made acid in reaction the plant might then become susceptible to curly top. Upon treatment of the plant with carbon dioxide, however, the reaction of the extracted juice of *C. murale* became more alkaline rather than more acid as was expected. Sugar beets were then treated with carbon dioxide and found to react in a similar way. The chemical studies and the probable reactions involved have been presented (6).[3]

Although the reaction in the cell sap resulting from carbon dioxide treatment was contrary to that expected, nevertheless the possible influence of this reaction on curly top resistance in sugar beets was investigated.

CARBON DIOXIDE TREATMENT OF SUGAR BEET SEEDLINGS

TREATMENT BEFORE AND DURING INOCULATION

In certain experiments beet seedlings were inoculated during their exposure to carbon dioxide. For these studies, a special type of chamber was required in order that the seedlings might be exposed to carbon dioxide and at the same time be inoculated by means of viruliferous beet leafhoppers, *Eutettix tenellus* (Baker), without subjecting the insects to the gas.

A tight box (fig. 1), with one side of glass, was constructed for the purpose of treating beet plants with carbon dioxide before and during inoculation. A beveled slit 1 inch wide at the top was cut the length of the lid. A strip of rubber sheeting was glued over the opening and

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securely held in place by thin strips of wood nailed on both edges.
A slit the same length as the opening in the lid was then cut in the
rubber. The edges of the rubber slightly overlapped, thus making
an almost airtight seal. A large number of seedlings in a crystallizing
dish were placed in the box and the tight-fitting lid clamped on. A
definite concentration of carbon dioxide was built up in the box and
maintained for a given period by admitting air and carbon dioxide at
a constant rate. The ratio of air to carbon dioxide, forced through
the chamber, was controlled by needle valves, with the aid of flow
meters that had been previously calibrated.

The plants were inoculated by drawing the tip of one cotyledon of
each plant through the slit in the rubber with a pair of rubber-tipped
forceps and placing on it a cage containing four viruliferous leafhop-
pers. The opening in the lid was long enough to provide space for
20 plants to be inoculated at the same time. The insects were caged
on the tip of the cotyledon and allowed to feed for one-half hour.
After the feeding period the cages were removed, together with those
portions of the cotyledons that were outside the gas chamber during
the feeding. The plants were allowed to fall to the bottom of the
chamber, where they remained in an atmosphere saturated with
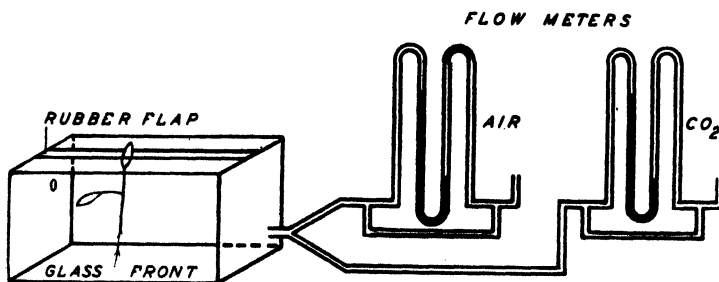
FIGURE 1.—Inoculating chamber and flow meters for controlling the gas mixture.

moisture until all the plants had been inoculated. After all the plants
in the box had been inoculated, they were removed and planted in
sand. Control plants were treated in the same manner, except that
they did not receive the carbon dioxide treatment.

TABLE 1.—*Percentage of curly top occurring on normal beet seedlings and on those
exposed to high concentrations of carbon dioxide immediately before and during
inoculation*

| | Plants | | Concentra-tion of CO_2 applied | Length of CO_2 treat-ment | Plants infected | |
Experiment no.	Check	Treated with CO_2			Check	Treated with CO_2
	Number	*Number*	*Percent*	*Hours*	*Percent*	*Percent*
1	64	63	38	4	75	19
4	32	80	28	2	47	9
5	131	127	20	2	12	0
6	108	100	25	2	31	9
8	117	100	60	3	36	10
9	36	20	40	3	17	0

The data in table 1 are typical of the results obtained in a large
number of experiments. It would appear from this table that the

plants subjected to carbon dioxide were rendered highly resistant to curly top. The ratio of the average percentage of infection in the controls to the average percentage of infection in the plants treated with carbon dioxide is 4.7 to 1. In some experiments the leafhoppers were allowed to feed on plants treated with carbon dioxide in darkness, while in others the plants were in the light during feeding. The percentage of infection resulting from these tests evidently was not affected by light.

Among the different experiments a wide variation was found in the degree of resistance to infection exhibited by the plants treated with carbon dioxide. Several factors probably contributed to bring about such a variation. In the first place, the concentration of carbon dioxide applied and the length of treatment were different for each experiment. It is possible also that the nutritional condition of the plants may have differed sufficiently to contribute to the observed variation.

It was found (6) that plants well nourished, especially with abundant nitrogen, responded to carbon dioxide treatment more quickly and to a much greater degree than slow-growing nitrogen-starved plants. The increase in pH values due to carbon dioxide treatment was much greater in the well-nourished plants. The nitrogen changes were also greater.

TREATMENT IMMEDIATELY AFTER INOCULATION

If the biochemical changes induced in the beet plant by carbon dioxide treatment before inoculation inactivate the virus or render it incapable of multiplication, it would appear logical to expect that treatment of the plant with carbon dioxide immediately following inoculation would reduce the amount of infection.

Experiments were therefore conducted to determine whether the virus could be inactivated by treating plants with carbon dioxide immediately following inoculation. Viruliferous leafhoppers were placed on seedlings for one-half hour, four leafhoppers per plant. One lot of the plants was immediately treated with carbon dioxide, while the untreated remainder served as controls. The plants were then transplanted to sand and the amount of infection was noted. It is evident from table 2 that the carbon dioxide treatment immediately following inoculation did not influence the percentage of plants infected and therefore that the treatment did not cause the plants to become resistant to the curly top virus. The average incubation period and also the severity of the disease on the plants treated with carbon dioxide were found to be the same as those observed on the control plants.

TABLE 2.—*Percentage of curly top occurring on normal beet seedlings and on those exposed to high concentrations of carbon dioxide immediately after inoculation*

Experiment no.	Plants		Concentration of CO_2 applied	Length of CO_2 treatment	Plants infected	
	Check	Treated with CO_2			Check	Treated with CO_2
	Number	*Number*	*Percent*	*Hours*	*Percent*	*Percent*
1	90	78	35	55½	92	90
2	131	29	20	4	10	14
3	98	80	20	32	.61	54

From the data shown it must be concluded that the biochemical changes that took place in the plant as a result of the carbon dioxide treatment after the plant had been inoculated were not important factors in preventing the establishment of the virus.

The question then arose as to whether the reduction in infection resulting from the treatment of the plants with carbon dioxide prior to and during inoculation (table 1) might have been due to a disturbance of the leafhopper's normal way of feeding.

In view of the magnitude of certain of the biochemical changes (6) brought about by the cells of the sugar beet when subjected to a high concentration of carbon dioxide, it seems logical that these or other chemical reactions that may take place may have a pronounced effect on the leafhopper. For example, the hydrogen-ion concentration of the juice of beet leaves treated with carbon dioxide was decreased in some cases to one-hundredth of its normal value; the ammonia nitrogen was found to more than double, while at the same time the amide nitrogen was reduced to one-half its normal value.

This theory is further supported by the fact that the leafhopper normally feeds in the phloem (1). and by the evidence presented by Bennett (1) which indicates that the insect must introduce the virus into the phloem in order to insure infection.

The extent to which these biochemical changes interfere with the leafhopper's normal way of feeding may be determined by observing in which tissue the leafhopper's mouth parts terminate.

FEEDING BEHAVIOR OF EUTETTIX TENELLUS

TISSUE FROM WHICH LEAFHOPPERS FEED WHEN CAGED ON NORMAL AND ON CARBON DIOXIDE-TREATED PETIOLES

Plants in approximately the six-leaf stage, having petioles 3 to 4 inches in length, were treated with approximately 50-percent carbon dioxide for 2 hours. The plants were then removed from the chamber, and leafhoppers were caged on the petioles for 20 minutes. After this treatment, the petioles from the carbon dioxide-treated and control plants were hand-sectioned and permitted to stand overnight in water. The sheaths left by the insects while feeding take on a yellow color if allowed to stand in water (1), so there was no difficulty in determining the tissue in which the leafhoppers had fed. The trails found, on microscopic examination, were classified according to the tissue in which they terminated and according to their position with respect to the phloem. Detailed results obtained from four experiments are presented in table 3.

When leafhoppers were forced to feed on the petioles of plants treated with carbon dioxide it was obvious that they lost their sense of direction and that their reaching the phloem was a mere matter of chance. From the data it would appear that the insects on the treated petioles were even unaware of their nearness to the phloem, as not one changed the direction of its mouth parts in an effort to reach this tissue. In contrast to this, in three of the four experiments a significant number of leafhoppers feeding on the controls changed the direction of their mouth parts in order to reach the phloem. Of the 95 feeding trails observed in the controls 7 were found to curve in the parenchyma tissue and, as a result, to terminate in the phloem.

TABLE 3.—*Disturbance of the normal feeding habit of the leafhopper when forced to feed on sugar-beet petioles treated with carbon dioxide*

Experiment no. and treatment	Punc-tures	Total punctures which—					
		Hit phloem direct	Curved in paren-chyma tissue to hit phloem	Reached phloem	Were deep enough but missed phloem	Were in direction to hit phloem but not deep enough	Were not in direc-tion to hit phloem and not deep enough
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1—Checks	23	47.83	8.70	56.53	0	17.39	26.09
CO₂ treated	22	4.54	0	4.54	9.09	36.36	50.00
2—Checks	21	57.14	0	57.14	9.52	19.05	14.29
CO₂ treated	13	15.38	0	15.38	7.69	30.77	46.15
3—Checks	28	46.43	14.28	60.71	3.57	28.57	7.14
CO₂ treated	33	12.12	0	12.12	21.21	42.42	24.24
4—Checks	23	43.48	4.35	47.83	0	39.13	13.04
CO₂ treated	31	16.12	0	16.12	9.68	16.12	58.06
Total—Checks	95	48.42	7.37	55.79	3.16	26.32	14.74
CO₂ treated	99	12.12	0	12.12	13.13	31.31	43.43

In view of the absence of probing, it appeared that the leafhoppers feeding on the petioles treated with carbon dioxide were satisfied as long as their mouth parts were inserted anywhere just beneath the epidermis. Of the total trails, 43 percent were not in the proper direction nor were they deep enough to reach the phloem. Only 31 percent of the trails were in the right direction to reach the phloem, but these were not deep enough. That is, approximately 74 percent of all the trails found in the treated petioles were not deep enough to reach the phloem, regardless of the direction in which they pointed.

In summing up the four experiments, it is seen that a total of 55.8 percent of the trails found in the untreated petioles terminated in the phloem, as compared to only 12.1 percent found in the phloem of the treated petioles. The ratio is 4.6 to 1. The striking agreement between this ratio and the ratio (4.7 to 1, table 1) of infection in normal plants to that in plants treated with carbon dioxide is further evidence to support that presented by Bennett (*1*), which indicates that the virus must be deposited in the phloem in order to insure infection.

FEEDING BEHAVIOR OF LEAFHOPPERS ON AN ARTIFICIAL FOOD ADJUSTED TO TWO DIFFERENT pH VALUES

Inasmuch as leafhoppers normally feed from the phloem, experiments were conducted to determine whether they prefer food that is alkaline rather than acid in reaction.

An artificial food consisting of 8 percent of sucrose in tap water was prepared. Sufficient sulphuric acid and a small amount of disodium phosphate were added to one-half of the liquid to stabilize the hydrogen-ion concentration at pH 5.0. The remainder of the liquid received the same amount of phosphate and sufficient sodium hydroxide to maintain the hydrogen-ion concentration at pH 8.5. To the under side of the membrane of a large cage, similar to that shown by Bennett (*2*) but 8.5 cm in diameter, were added large drops of the acid and of the alkaline food in three alternate rows of 6 drops each, making a total of 36 drops. Twenty-five leafhoppers were placed in the cage. The

numbers of leafhoppers sitting over the acid and over the alkaline drops were noted at the end of each 5-minute interval for 50 minutes.

Two similar experiments were conducted, in which the membranes of two cages, 8.5 and 4.0 cm in diameter, respectively, were marked off in quadrants. Sufficient agar-agar was added to the artificial food to produce a solid medium when poured in thin layers on plate glass. Sections of the solidified food were cut the size of the quadrants and pressed on the membranes, the acid food being placed on opposite quadrants. Twenty-five leafhoppers were placed in each cage and left unmolested. The number of leafhoppers sitting over the acid and over the alkaline food were counted at the end of each 5-minute interval for 40 minutes. The data obtained in the three experiments are presented in table 4.

TABLE 4.—*Number of leafhoppers feeding on an artificially prepared food adjusted to 2 different pH values*

Experiment no.	Arrangement of food on membrane of cage	Length of feeding period	Average number of leafhoppers collecting over food at end of each 5-minute interval	
			Alkaline food (pH 8.5)	Acid food (pH 5.0)
		Minutes	*Number*	*Number*
1	In drops (36), in alternate rows of acid and alkaline food	50	7.9	2.0
2	Quadrants (membrane 8 cm in diameter), with acid food on opposite quadrants.	40	7.4	2.3
3	Quadrants (membrane 4 cm in diameter), with acid food on opposite quadrants.	40	7.0	2.9

It is evident that the leafhoppers concentrated on the food that was alkaline in reaction. They were observed, however, to explore the drops and quadrants of acid food by puncturing several times in different places and then to move on.

It was evident from observing the leafhoppers on the acid food, as recorded in table 4, that they were not feeding but were merely exploring the area.

Leafhoppers were also allowed to feed on single drops of solid media, one-half of which was acid and the other half alkaline. Such drops were formed as follows. To the melted media was added sufficient bromthymol blue to distinguish the acid from the alkaline medium. One large drop of the acid and one of the alkaline medium were deposited on a membrane, being separated only by a thin cover glass. The cover glass was removed when the media were partly solidified, and in this way direct contact was made between the acid and the alkaline food.

When leafhoppers were allowed to feed on such drops they explored the entire drop and then invariably fed from the alkaline side. The mouth parts could be seen in the alkaline food with the aid of a hand lens.

From the experiments presented thus far it is evident that the carbon dioxide treatment causes such striking chemical changes to take place in the beet plants that the leafhoppers are no longer able

to find the phloem. It is also evident that the leafhoppers prefer an alkaline food to one that is acid in reaction.

The implication is that under normal conditions the leafhoppers are guided to the phloem by a chemical gradient and that this gradient is so profoundly affected by the carbon dioxide treatment that in treated plants it is no longer present to direct the leafhoppers to the phloem; consequently, the insects are unable to find the phloem except by chance. As a result only an occasional infection is produced (table 2).

A consideration of the facts presented led to the hypothesis that this chemical gradient is in reality a pH gradient and that it is upset by treating the plant with a high concentration of carbon dioxide.

The evidence that led to these hypotheses is as follows: (1) The phloem contents of the beet petiole are alkaline while the cell sap of the parenchyma is acid in reaction; (2) the saliva of the leafhopper has been found to be alkaline in reaction (5) (which is significant, because the leafhopper must penetrate acid tissue in order to reach the alkaline phloem on which it feeds; in other words, the leafhopper's mouth parts are in an extremely sour medium as compared with its own saliva); (3) on treatment with high concentrations of carbon dioxide, the reaction of the parenchyma becomes more alkaline (6), while the phloem content becomes less alkaline or even acid, as will be shown later; (4) such a change in the reaction of the parenchyma and the phloem would upset any pH gradient that might exist; (5) the majority of the trails left by the leafhoppers while feeding on the normal petiole terminate in the phloem tissues; (6) the leafhoppers even changed the direction of their mouth parts in the parenchyma in order to reach the phloem in normal plants (table 3; Bennett (1) has observed this to be a very common occurrence); (7) only a very small percentage of the trails left by the leafhoppers while feeding from petioles treated with carbon dioxide terminated in the phloem tissues; (8) not one of the trails found in the treated petioles indicated that any leafhopper had changed the direction of its mouth parts in the parenchyma in order to feed from the phloem; (9) the leafhopper prefers an alkaline food to one that is acid in reaction (table 4).

THE pH GRADIENT IN NORMAL AND IN CARBON DIOXIDE-TREATED SUGAR-BEET PETIOLES

To test the hypotheses that the leafhoppers are guided to the phloem by a pH gradient and that this gradient is upset by carbon dioxide treatment, a microquinhydrone electrode was devised to measure the pH value of the cell sap of individual cells. Such measurements would reveal the nature of the pH gradient from the phloem to the epidermis of the petiole in the sugar-beet petiole.

A platinum needle sealed into a 3-mm glass tube was sharpened first by means of a file, then a hone, until a sharp, gradually tapering point was obtained. The sharpening was completed by placing the needle on a hone, where it was stroked (toward the point) with a rubber eraser containing powdered pumice. By this procedure a needle was made having a gradual taper to the point, which was 40μ in diameter.

The average diameter of the cells of the parenchyma of large beet petioles of the type used were found to be 150μ with only 10 percent

falling below 100μ. Consequently, the microelectrode could be inserted into a single cell without rupturing or disturbing the adjoining cells.

The microelectrode was clamped in one of the arms of a micromanipulator, making movement in any direction possible. A large freshly cut petiole, 3 inches in length, was mounted in a perpendicular position in the other arm of the micromanipulator. The lower end of the petiole was immersed in a saturated solution of potassium chloride, which served as a salt bridge and was in turn connected to a standard half cell. A dissecting microscope was mounted in front of the micromanipulator at an angle of approximately 25° from the perpendicular to allow the microelectrode to be mounted in a perpendicular position. The microscope was focused on the surface cells of the section of the petiole, and the tip of the microelectrode was placed
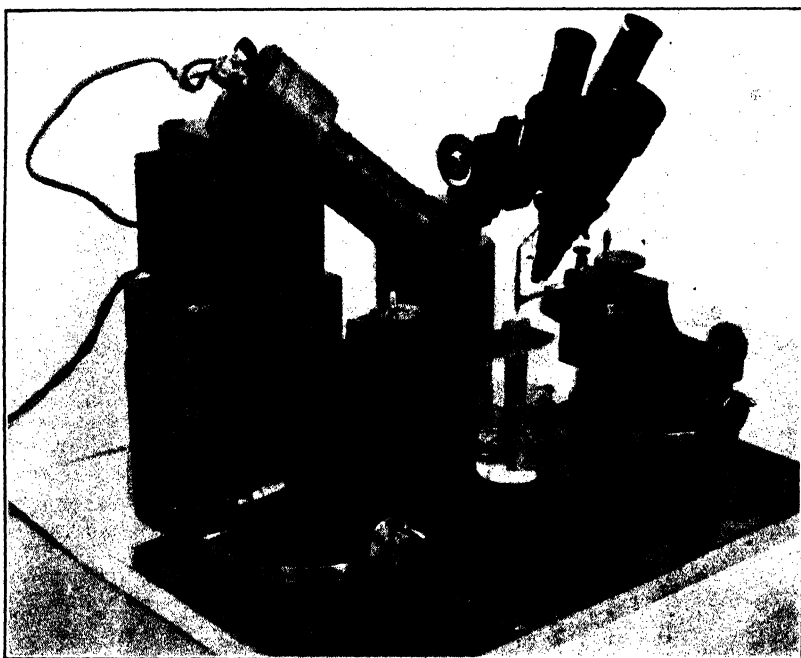
FIGURE 2.—Apparatus used to measure the pH value of the cell sap of individual parenchyma cells of sugar-beet petiole.

in the center of the field of view and near enough to the surface of the petiole to be in focus also. The apparatus described is shown in figure 2.

To determine the pH value of any cell or tissue of the petiole a fresh surface was cut, washed with distilled water, and dried with a filter paper. The microscope was focused on the surface cells of the petiole, which was then moved until the desired cells were in the microscope field. A minute amount of finely ground quinhydrone was sprinkled with a small camel-hair brush on the area to be tested. With the tip of the microelectrode in the center of the field and also in focus, the petiole was then moved until the desired cell was directly

below the microelectrode. The microelectrode was then lowered until it punctured the cell wall. The cell sap, being under slight pressure, forced its way past the microelectrode, thereby filling the cup formed by the cut cell above in which the quinhydrone was deposited. In order to get the quinhydrone into the punctured cell it was only necessary to withdraw the microelectrode. The particles of quinhydrone, being wet by the cell sap, could be seen to sink through the hole made by the microelectrode. The microelectrode was then lowered and the potential measured.

Because of the high resistance offered by the petiole it was necessary to employ a very sensitive galvanometer to measure the potential difference. The galvanometer used had a sensitivity of 0.00097 microampere per millimeter (scale at 1 m).

It required approximately 3 minutes from the time a fresh section was cut to determine the pH gradient, starting from the phloem and going to the epidermis of the petiole, or the reverse. The pH gradient was determined in different sections of the same petiole. Measurements were also made on a large number of petioles collected on different days.

The curve of figure 3 is typical of the measurements made of the pH gradient in the petioles of the normal sugar beet.

FIGURE 3.—The pH gradient in normal sugar-beet petiole: *xy*, Xylem; *ph*, phloem; *sh*, bundle sheath; *pa*, parenchyma; *ep*, epidermis

The pH gradient was also measured in petioles that had been subjected to 75-percent carbon dioxide for 16 hours and in petioles that had been treated with the gas and allowed to recover (fig. 4).

The pH value of the individual parenchyma cells was found to differ only slightly from that of the extracted juice as measured by the glass electrode. For example, by means of the glass electrode the mean pH value of the parenchyma beyond the fifth cell from the phloem tissue was found to be 5.9 and the pH value of the extracted juice of the same lot of petioles was found to be 5.7. A considerable number of pH measurements on the phloem exudate by colorimetric and potentiometric methods have been made. A pH value of approximately 8 was found in every case. Inasmuch as the electrode

crushes a small number of cells (among which are phloem parenchyma cells) when inserted into the phloem tissue, the pH values obtained are low as compared to the pH value of the contents of the sieve tubes. It is very probable that the pH value of the cell sap of the phloem parenchyma is considerably lower than that of the sieve-tube contents.

It is evident that a definite gradient exists out to the fourth or fifth parenchyma cell, the maximum change in pH value being about 2 units, or a hundredfold increase in the hydrogen-ion concentration, going from the contents of the phloem to the cell sap of the parenchyma cells near the epidermis.

The hydrogen-ion concentration of the phloem contents (fig. 4, *A*) has been increased approximately a hundredfold (from a pH of 8.2 to 6.2) by carbon dioxide treatment. The normal pH gradient has been entirely upset and even reversed.

The reversal of the gradient is probably due to the amount of carbon dioxide absorbed by the cells. The partial pressure of carbon dioxide would be greater in the cells immediately below the epidermis; consequently the reactions (*6*) producing hydroxyl ions would proceed further. The cells near the vascular tissue, far removed from the source of carbon dioxide, would alter their hydrogen-ion concentration but little. In this way the pH gradient may for a short period even be reversed.

FIGURE 4.—*A*, pH gradient in a sugar-beet petiole treated with carbon dioxide; *B*, pH gradient in a recovered petiole, after carbon dioxide treatment. *xy*, Xylem; *ph*, phloem; *sh*, bundle sheath; *pa*, parenchyma; *ep*, epidermis.

If the pH gradient is the means by which the leafhoppers are guided to the phloem, then it is clear why such a large percentage of the trails in the petioles treated with carbon dioxide terminated in the parenchyma immediately below the epidermis. This is the point at which the hydrogen-ion concentration is least. If the leafhoppers penetrated deeper, the hydrogen-ion concentration would become greater instead of less, as in the normal plant.

It may be pointed out that in the cases where the leafhoppers changed the direction of their mouth parts in the parenchyma in order to feed from the phloem, they began changing direction approximately at the third or fourth cell from the bundle sheath. The

evidence strongly indicates that it is the pH gradient which guides the leafhoppers to the phloem in the normal plant.

DISCUSSION

In view of the alkaline reaction of the saliva of the leafhopper and the acid reaction of the parenchyma of the beet plant, it seems logical that a gradual pH gradient from the epidermis to the phloem would be instrumental in directing the leafhopper to the phloem. When the leafhoppper punctures the first cells below the epidermis, an enormous difference in hydrogen-ion concentration exists between the alkaline saliva of the leafhopper and the acid cell sap of the parenchyma. For example, if the pH value of the leafhopper's saliva is assumed to be 8.5 and that of the cell sap of the parenchyma to be 5.8, the cell sap taken up by the leafhopper would have a hydrogen-ion concentration 800 times greater than the saliva of the leafhopper. It seems probable that such a difference would have a pronounced physiological effect upon the leafhopper.

As the leafhopper's mouth parts penetrate deeper into the parenchyma, the difference in hydrogen-ion concentration decreases slowly. When the mouth parts approach to within three or four parenchyma cells of the bundle sheath (where the change in the pH gradient becomes very marked), the difference between the hydrogen-ion concentration of the cell sap and that of the saliva decreases rapidly. When the mouth parts reach a sieve tube, the difference between the hydrogen-ion concentration of the sieve-tube contents and that of the saliva becomes negligible, a condition preferred by the leafhopper.

Brandes (*3*), Davidson (*4*), Horsfall (*7*), and Smith (*13*) have shown that certain species of *Aphis* feed from the phloem tissues, but no explanation of the mechanism which directs the insect to these tissues has been offered.

It is quite evident from these investigations that it is no mere matter of chance that so large a proportion of the leafhoppers reach the phloem while feeding on the normal beet plant.

The idea of a pH gradient is not new. Sachs (*11*), in 1862, demonstrated that the phloem contents were alkaline to litmus.

Pearsall and Priestley (*8*) found the pH value of the xylem to be from 4.3 to 5.0 and, recalling that Sachs pointed out that the phloem may be as high as 7.8 or higher, remarked that the two cambial meristems of the normal dicotyledon lie across a marked pH gradient of hydrogen-ion concentration. The generalization was made that cambial activity takes place where there is a pH gradient, as from acid xylem to alkaline phloem. No measurements were made, however, to show the type and extent of the gradient that exists.

Priestley (*9*) discussed the general occurrence of phellogen, vascular cambium, and apical meristems and the origin of adventitious apical meristematic organizations in the light of the existence of such a pH gradient.

Rea and Small (*10*) and Small (*12*) made a very extensive general survey of tissue reactions by the range-indicator method. This method at best can only indicate roughly the general range in which the different tissues lie. With this method these investigators pointed out that the general pH gradient between phloem walls and xylem walls varies from 0.4 to 1.5 pH units in range.

Most of the modern theories attempting to explain growth and the metabolic processes of individual cells or tissues recognize the vital importance and influence that the hydrogen-ion activity may exert on the dynamic forces operating within the cell.

The unique mechanism the cells have for altering the hydrogen-ion activity of their own cell sap in the presence of high concentrations of carbon dioxide may serve admirably in studying certain of the fundamental factors involved in the metabolic processes within the cell and meristematic activity.

SUMMARY AND CONCLUSIONS

A study has been made of the relation of cell-sap reaction to curly top resistance in the sugar beet. As preliminary studies indicated a possible relation between hydrogen-ion concentration and the disease, sugar beets were treated with carbon dioxide.

Only a small percentage of the sugar-beet seedlings became infected with curly top when exposed to a high concentration of carbon dioxide preceding and during the inoculating period. The ratio of infection in the normal plants to that in the plants treated with carbon dioxide was found to be 4.7:1. Exposure of sugar-beet seedlings to high concentrations of carbon dioxide immediately after inoculation failed to reduce the percentage of infection below that of untreated controls.

When the curly top vector, *Eutettix tenellus* (Baker), feeds on the petioles of sugar-beet plants treated with carbon dioxide, it apparently loses its sense of direction, and its reaching the phloem, from which the insect normally feeds, is then a mere matter of chance. Fifty-six percent of the trails observed in the normal petioles terminated in the phloem, whereas only 12 percent of the trails found in the treated petioles terminated in this tissue. The ratio is 4.6:1.

The striking agreement between this ratio and the one noted in the second paragraph of this summary is further evidence that the virus must be deposited in the phloem in order to insure infection. The curly top vector, *Eutettix tenellus*, prefers an alkaline food (pH 8.5) to one that is acid in reaction (pH 5.0).

A microquinhydrone electrode was built in such a way that the pH value of the individual parenchyma cells of the sugar-beet petiole could be measured without disturbing adjoining cells.

A gradual pH gradient was found in the normal petioles of the sugar beet. By exposing beet petioles to high concentrations of carbon dioxide, the normal pH gradient was entirely upset and even reversed. The pH gradient in the petioles treated with carbon dioxide returned to normal when placed under atmospheric conditions.

The evidence indicates that leafhoppers feeding under normal conditions are probably guided to the phloem by the pH gradient.

Evidence of what appeared at first to be curly top resistance induced by pH changes in the host revealed that the apparent resistance of the beet plants could be attributed to the failure of the vector to inoculate plants that were under the influence of carbon dioxide. The carbon dioxide treatment upsets the normal pH gradient in the beet plant, which, evidence strongly indicates, is the means by which the leafhopper is able to locate the phloem.

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FURTHER STUDIES ON THE RELATION OF THE CURLY TOP VIRUS TO PLANT TISSUES [1]

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INTRODUCTION

As outlined in previous papers (*2, 5, 6, 7*),[2] considerable evidence has been obtained that shows a very close relationship between the curly top virus and the phloem tissue of affected plants. This evidence is based on (1) anatomical studies that indicate restriction of necrosis to the phloem, (2) results of ringing experiments with *Nicotiana tabacum* L. and *N. glauca* (Graham) that indicate inability of the virus to pass an area devoid of phloem, and (3) determinations of relative amounts of virus picked up by the insect vector, *Eutettix tenellus* (Baker), when forced to feed on different tissues of the beet plant.

Improvement in methods for the recovery of virus from plant tissues has permitted a more extensive study of the virus content of different types of tissues and has provided a means of verifying the accuracy of previous determinations in which the virus content of tissues was measured by the amount of virus the insect vector was able to obtain by direct feeding. The results of some further studies on the relation of the curly top virus to different organs and tissues of beet, *Beta vulgaris* L., and tobacco, *Nicotiana tabacum*, are presented in the following report.

In these studies a special effort was made to obtain additional evidence bearing on the extent to which the curly top virus is restricted to the phloem tissue of affected plants.

MATERIAL AND METHODS

The beet plants used in these studies were of the curly top resistant variety U. S. 33. Seeds were planted in the fall of 1933 and 1934 and the tests were made on nearly mature plants in July, August, and September, of 1934 and 1935. Infection was from field sources but plants selected for the study were infected prior to the beginning of seedstalk formation, as indicated by the presence of symptoms on leaves below the base of the seedstalks. The symptoms on these resistant plants were relatively mild, and the disease did not markedly interfere with plant development. Numerous experiments have indicated that the concentration of recoverable virus in plants of this type is somewhat lower than that in plants injured more severely. However, the recovered virus was of a virulent type and produced severe symptoms on seedling beets of susceptible varieties.

The tobacco plants selected for study were of the Turkish variety. They were grown under greenhouse conditions and inoculated wit

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stock strains of the curly top virus. With only a few exceptions, organs and tissues that developed after the plants became infected were selected for tests.

The method employed in the tests of virus content of various organs and tissues of these plants was described in a previous publication (*3*). Juice was expressed from the tissues to be tested and added to an equal volume of 95-percent alcohol. The resulting precipitate was thrown down by centrifugation, washed once with 50-percent alcohol, dried, mixed with a volume of 5-percent sugar solution equal to the original volume of the juice, and centrifuged, and the supernatant liquid was used to feed nonviruliferous leafhoppers. After a feeding period of 4 to 6 hours, the leafhoppers were caged singly on beets for 7 days, and the number of plants infected by these leafhoppers was taken as a relative measure of the virus content of the tissue from which the original juice was derived. The preparations, regardless of the original source of the juice, provided a relatively uniform food material for the beet leafhopper. This method was used for all parts from which juice could be extracted. For certain structures, such as seeds and pollen, modifications of the method were introduced. These are described in later sections of this paper.

From both beet and tobacco, representative samples of the organs and tissues tested for virus content were selected for histological studies. These samples were killed in a chromo-acetic-formalin solution (*5*) and embedded in paraffin after dehydration in ethyl alcohol and clearing in butyl alcohol.

STUDIES ON SUGAR BEET

RELATION OF THE VIRUS TO TISSUES OF VEGETATIVE PARTS

In a consideration of the relation of the curly top virus to the plant, three types of tissues seem of major importance: (1) Meristem, (2) phloem, and (3) parenchyma. Studies on the distribution and concentration of virus in the plant are limited (1) to observations on occurrence and nature of pathologic changes indicating presence or absence of virus in different tissues and (2) to a recovery of the virus from these tissues by various means. As yet, not much evidence has been obtained on the relation of the curly top virus to meristem, but studies on parenchyma and phloem have been more successful.

The curly top virus occurs in the phloem in high concentrations. Evidence indicates a much lower concentration in parenchyma. Undoubtedly a large part of the virus that occurs in parenchyma is present in the exudate that escapes into the intercellular spaces of this tissue from the phloem. The extent to which the virus is able to invade parenchyma and the relation of the virus to the protoplasm of parenchymatous cells are of special interest in the case of curly top because of the apparently close relationship of the causal agent to the phloem tissue of affected plants.

ANATOMICAL OBSERVATIONS

Following infection, the phloem develops striking abnormalities leading in most instances to necrosis. In susceptible varieties phloem necrosis is very pronounced (figs. 1, 2), but in the resistant plants used in this study this symptom was very mild (fig. 3, *B*) or was absent entirely.

Susceptible plants usually show severe stunting of the various organs, a phenomenon indicatinghy poplastic condition of tissues. Resistant plants, however, show relatively little stunting, and frequently in very resistant individuals no stunting is evident.

Whether or not the diseased plants show hypoplasia, the parenchyma of the pith of the crown and stalk and the ventral parenchyma of the petiole seem to develop no cytological abnormalities that could be taken as an indication of presence of virus in these regions. Examination of living cells and of material treated with different killing
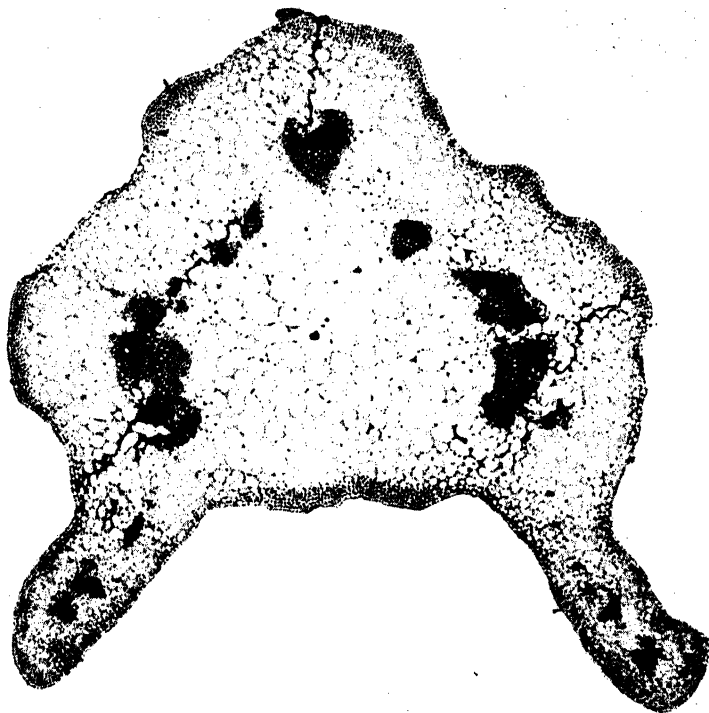
FIGURE 1.—Transverse section of a sugar-beet petiole from a plant severely affected by curly top. The phloem appears near the dorsal side of the petiole and shows severe necrosis accompanied by leakage of material to the surface of the petiole. × 23.

agents, including certain of those employed in mitochondrial technique, revealed no consistent differences between parenchyma cells of healthy and diseased plants.

In the leaves of severely affected susceptible plants, phloem necrosis is quite extensive and is frequently accompanied by exudation of material from the diseased phloem into intercellular spaces of the adjacent parenchyma and even to the exterior of the petiole and blades (fig. 1). When exudation is very abundant, the cells lying along the path of the material may separate from each other along the middle lamella (fig. 1, right), or may hypertrophy (fig. 1, above and left). Sometimes the exudate is not so abundant and fails to affect
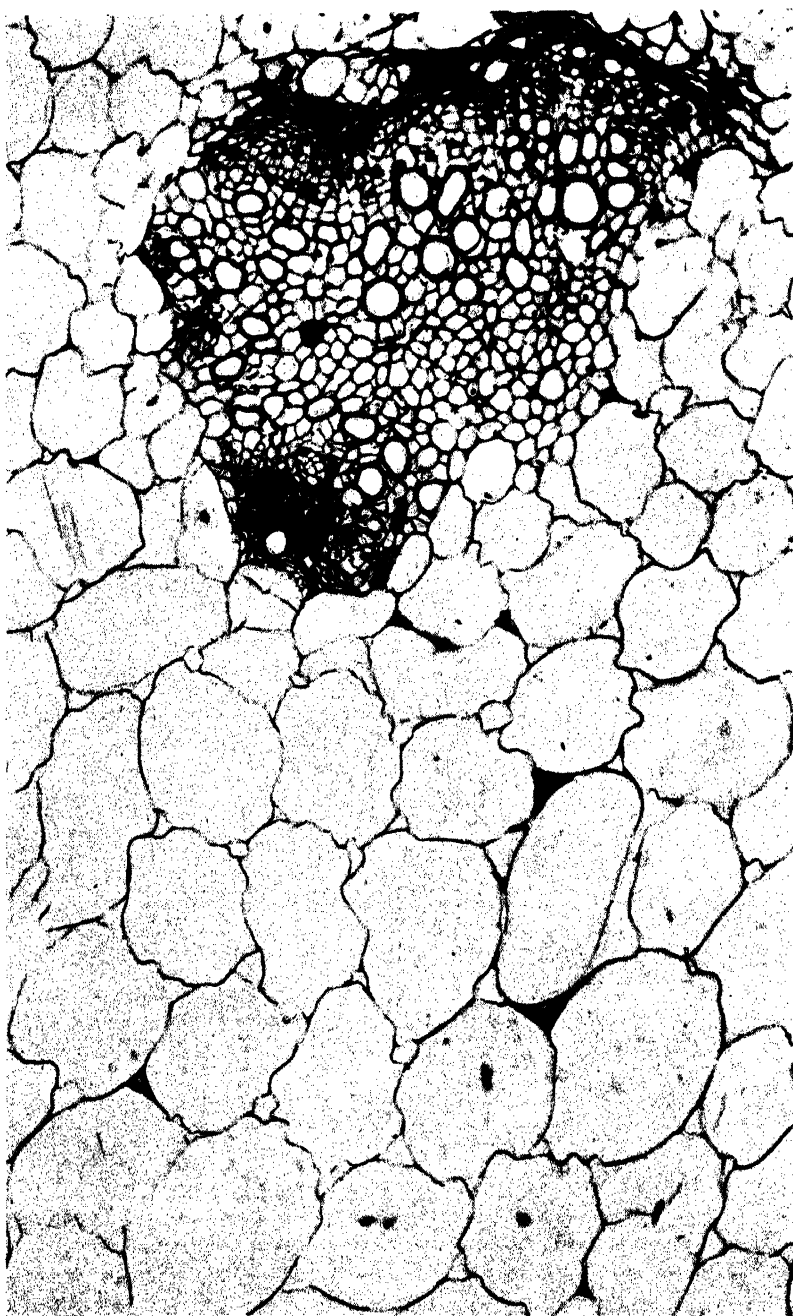
FIGURE 2.—Transverse section of a vascular bundle with adjacent ventral parenchyma from a diseased sugar-beet petiole. The bundle has phloem on both sides of the xylem. Black-stained exudate from the internal phloem group fills some of the intercellular spaces of parenchyma. × 150.
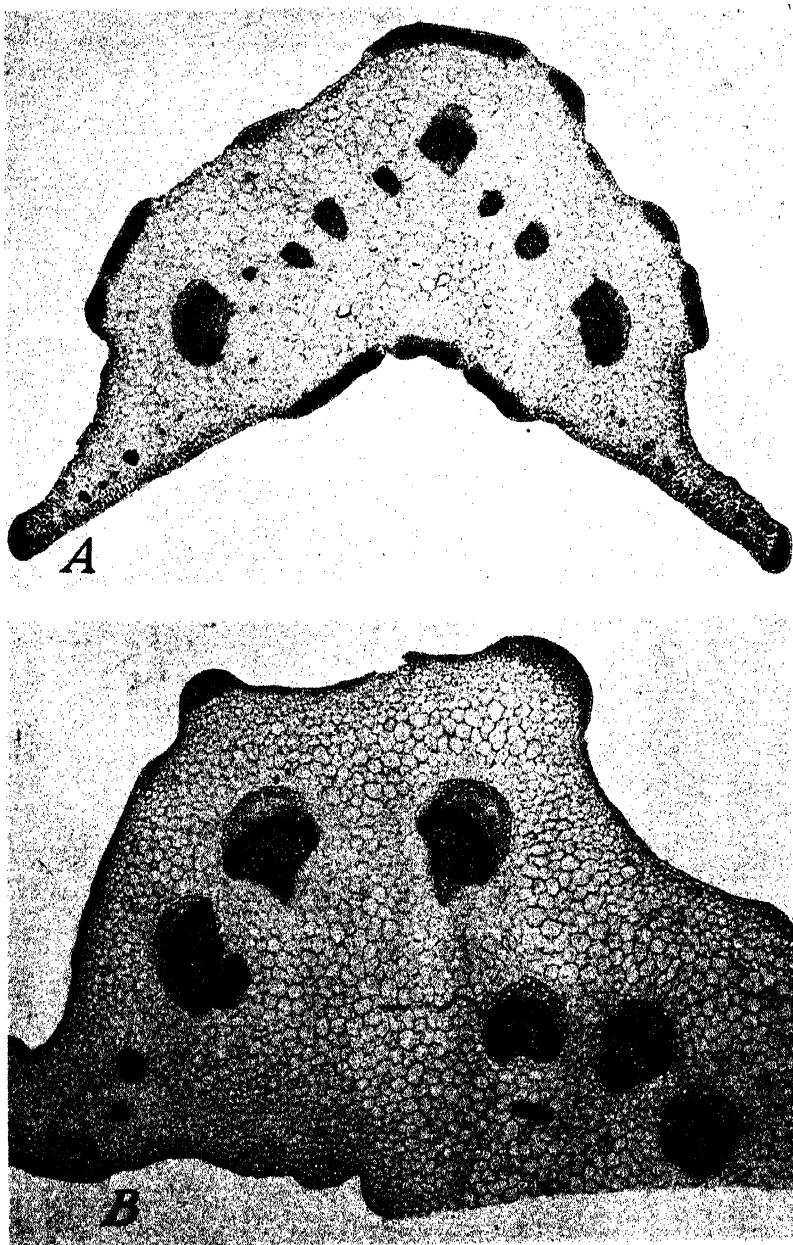
FIGURE 3.—*A*, Transverse section of a healthy sugar-beet petiole; *B*, transverse section of a portion of a petiole from a beet affected by curly top. Both petioles are placed with their dorsal sides above. Each vascular bundle shows xylem pointing toward the ventral side of the petiole, phloem and bundle cap toward the dorsal side. *B* shows some phloem necrosis. Both × 12.

the position and size of the cells between which it forces its way, but fills the intercellular spaces with darkly staining material (fig. 2). In the petiole most exudate moves from the phloem toward the periphery on the dorsal side, but some of it appears also in the intercellular spaces of the ventral parenchyma. This is particularly common near the extreme ends of the semicircle of bundles. Here the phloem swings around the xylem so that the bundle becomes bicollateral. Exudate from the internal phloem in figure 2 has moved into the intercellular spaces of the ventral parenchyma. The outer phloem, however, may also furnish some of the exudate that occurs on the ventral side. The presence of exudate in the ventral parenchyma of the petiole may be seen in figures 1, 2, and 4.
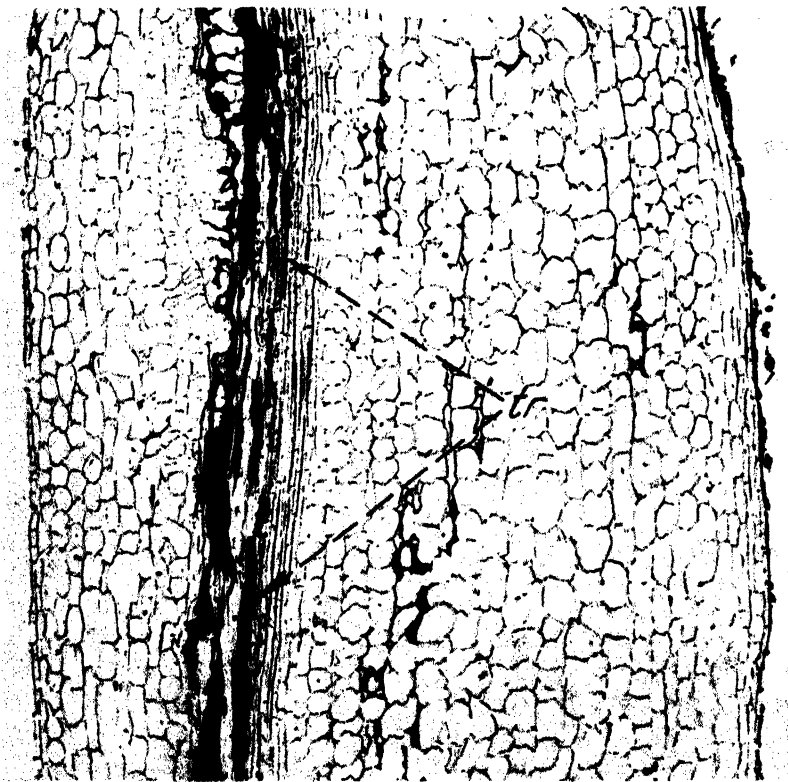
FIGURE 4.—Longitudinal section of a petiole from a diseased sugar beet. To the left is a vascular bundle with necrotic phloem. Within the xylem two sections of tracheae (tr) are filled with black-stained phloem exudate. The ventral parenchyma to the right of the bundle also contains such exudate in the intercellular spaces. × 46.

In resistant varieties of beets movement of material from the phloem into the intercellular spaces of the neighboring parenchyma is much less frequent than in susceptible varieties. When this occurs at all, the volume of material that moves into the parenchyma is relatively small and is confined, so far as observed, to areas in the leaf blade.

Infected plants of the resistant variety U. S. 33 were examined microscopically for the presence of phloem exudate in the intercellular

spaces of parenchyma of different parts of the plants. No exudate was found in the intercellular spaces of the parenchyma of the crown and flower stalk or in the ventral parenchyma of the petiole (fig. 3, *B*). It is not known that phloem exudate can be detected by microscopic examination in all cases, but, on the basis of the available evidence, it is considered that probably the parenchyma of selected individuals of this variety is as free from intercellular exudate as that of any other variety that might be selected.

TESTS FOR VIRUS

The most favorable plant parts from which to obtain tissue free from phloem are the pith of the crown (fig. 5, *A*), parenchyma of the ventral side of large petioles (fig. 5, *B*), and pith of the flowering stalk.
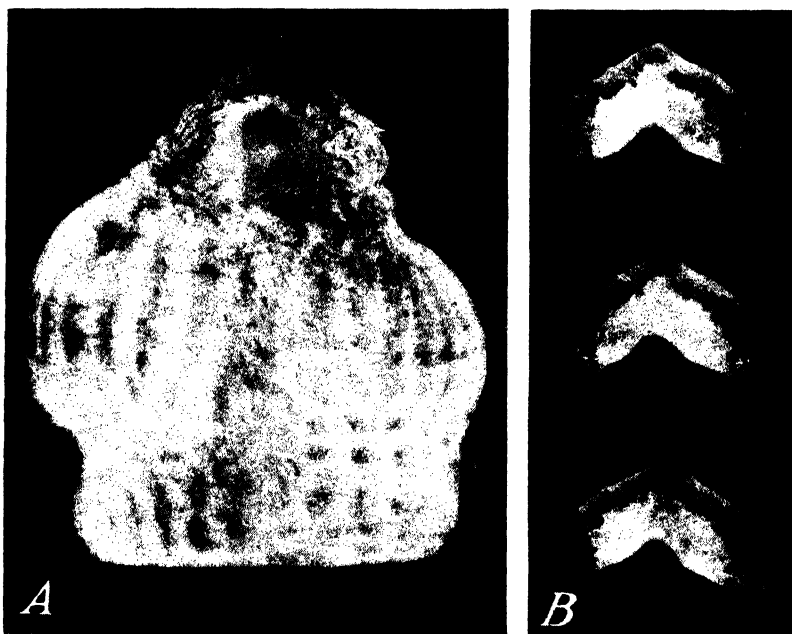
FIGURE 5.—*A*, Longitudinal section through the crown of a sugar beet showing the pith region above, which is free of vascular tissue; *B*, transverse sections through large sugar-beet petioles showing the vascular bundles near the periphery on the dorsal side of the petiole and a large parenchymatous area, free of vascular tissue, on the ventral side. × 1.

Vigorous plants with large petioles and fruitstalks were selected from field plots. The symptoms consisted mainly of vein swellings and indicated that the plants had a high degree of resistance. Blocks of parenchyma were removed from the crown, flowering stalk, and petioles of these plants. Some of these blocks were examined microscopically by means of freehand and prepared sections. No morphological or cytological abnormalities were observed and no phloem exudate was discovered in the intercellular spaces of the tissue of these regions.

Other blocks of parenchyma were removed and washed thoroughly in distilled water. The juice was expressed and its virus content was determined as already described. This virus content was compared

with that of equal volumes of juice from adjacent regions containing vascular tissue.

The results of these tests are shown in experiment 1 of table 1. They are in general similar to those obtained by feeding the leafhoppers directly on the tissues, and they support the concept that the curly top virus is much more concentrated in the phloem than in the parenchyma of the regions tested. No virus was obtained from the parenchyma of the petiole and in 20 of the 26 tests none was recovered from the parenchyma of the crown and flowering stalk. In the tests in which virus was recovered from parenchyma, its concentration appeared very much lower than in adjacent regions containing vascular tissue.

TABLE 1.—*Virus content of different organs and tissues of sugar-beet plants*

Experiment no.	Source of virus	Tests[1]	Tests that yielded virus	Plants inoculated	Plants infected	
		Number	*Number*	*Number*	*Number*	*Percent*
	Leaf	10	10	200	64	32.0
	Parenchyma of petiole	10	0	200	0	0
	Adjacent tissue	10	5	200	13	6.5
	Parenchyma of crown	12	1	240	5	2.0
1	Adjacent tissue	12	12	240	60	25.0
	Parenchyma of crown	6	2	120	3	2.5
	Adjacent tissue	6	6	120	56	46.6
	Parenchyma of stalk	8	3	160	3	1.8
	Adjacent tissue	8	8	160	47	29.3
2	Pericarp	16	14	320	59	18.4
	Seeds (mature)	16	16	320	205	64.0
3	Embryo	8	0	160	0	0
	Perisperm and seed coat	8	8	160	70	43.7
	Seeds	4	4	80	37	46.2
4	Seeds minus vascular region	4	4	80	17	21.2
	Vascular region of seeds	4	4	80	31	38.7
	Seeds	7	5	140	18	12.8
5	Upper half of seeds	7	1	140	1	.7
	Lower half of seeds	7	5	140	11	7.8
	Nonfilled seed coats	3	0	60	0	0
6	Embryo (germinated)	3	0	60	0	0
	Perisperm and seed coat	3	2	60	17	28.3
	Pericarp	6	5	120	18	15.0
7	Seeds (young)	6	1	120	1	.8
	Seeds (medium)	3	2	60	16	26.6
	Seeds (mature)	6	6	120	77	64.1

[1] 20 plants were inoculated in each test.

RELATION OF THE VIRUS TO FLOWER AND FRUIT PARTS

Symptoms of curly top are evident on the calyx, stamens, and pistils of flowers from infected plants. The external symptoms consist mainly of enlarged veins. Phloem degeneration occurs in all parts of the flower, hypertrophy and hyperplasia being evident as soon as sieve tubes are mature.

Unfortunately, the beet flower is too small to permit tests of its parts for relative virus concentration by the methods employed, so that little more than anatomical evidence is available regarding distribution of the virus in the flower parts. This evidence, however, indicates that the virus occurs in all the vascular bundles of the flower.

The beet seed is of considerable size and large enough to permit tests for virus content of its parts. Rather extensive tests were made to determine relative concentration and distribution of virus in the seed. In conjunction with these tests, anatomical studies were made to gain

an understanding of the normal anatomy of the mature seed and of the effect of the disease on the cytological and anatomical structure of the different seed parts.

Normal and Morbid Anatomy of the Seed

The sugar-beet seed is a flat lentil-shaped structure 1.5 to 2 mm in diameter. It lies horizontally in the ovarian cavity and the funiculus is bent under it. When a mature seed is removed from the green pericarp the funiculus is still intact and appears on the lower side of the seed as a small sharply bent stalk (fig. 6, *A*, *B*).

The embryo is curved around the perisperm, a starchy storage tissue of nucellar origin. In the mature seed only a small amount of endosperm occurs near the radicle (figs. 6, *C*; 7).

The embryo is rich in albuminous and oily storage materials. According to De Vries (*9*), starch is absent from the resting embryo but appears there during germination, when the embryo absorbs starch from the perisperm.

The seed coat has two layers of thick-walled cells enclosing between them some thin-walled parenchyma (figs. 7, 8). In the regions where the seed coat is thinnest only one or two layers of parenchyma cells occur, but near the chalaza they are more numerous. The vascular tissue of the seed is distributed within the parenchyma of the chalazal region.

The vascular tissue is composed of primary xylem and phloem. The strand that connects the ovule with the mother plant passes through the funiculus and enters the parenchyma lying between the inner and outer layers of the seed coat. Approximately midway between the outermost limit of the outer integument and the inner limit of the inner integument, the strand splits in two and forms a saddle-shaped structure which embraces the base of the perisperm (figs. 6, *A*, *B*, *D*; 8). The region traversed by the two branches of the funicular strand is referred to as the chalaza in this paper.

Within the funiculus proper the vascular strand is collateral (fig. 6, *E*), but within the chalaza and slightly below it the phloem occurs on all sides of the xylem (fig. 6, *F–H*). The phloem extends farther than the xylem, so that at the summits of the branches only phloem elements appear in section (fig. 6, *I*).

There is a heavy cuticle on the inner wall of the innermost layer of the seed coat. This cuticle may be identified by the use of Sudan III, when the embryo consists of only about nine cells. The cuticle stops abruptly in the chalazal region where the phloem approaches the perisperm (figs. 7, 8). In this manner a "passage region" is left between the chalaza and the perisperm through which materials may move from the phloem into the perisperm. The phloem, however, does not come in direct contact with the perisperm cells. A tightly packed layer of cells, remarkably rich in tannin compounds, intervenes between the phloem and the perisperm (figs. 6, *C–I*; 7; 8). The corners of these cells have prominent thickenings which show the characteristic fatty staining reaction with Sudan III. The deposition of this fatty substance occurs at the time the cuticle is laid down on the inner wall of the inner integument. When the seed is mature and the funiculus is dried up, the entire walls of the passage region show positive reaction with Sudan III. The embryo and perisperm appear to become sealed off in a suberized layer.

As in all other seeds, vascular connection does not exist between the embryo and the mother plant. But the embryo itself develops mature protoxylem elements in the cotyledons before the seed
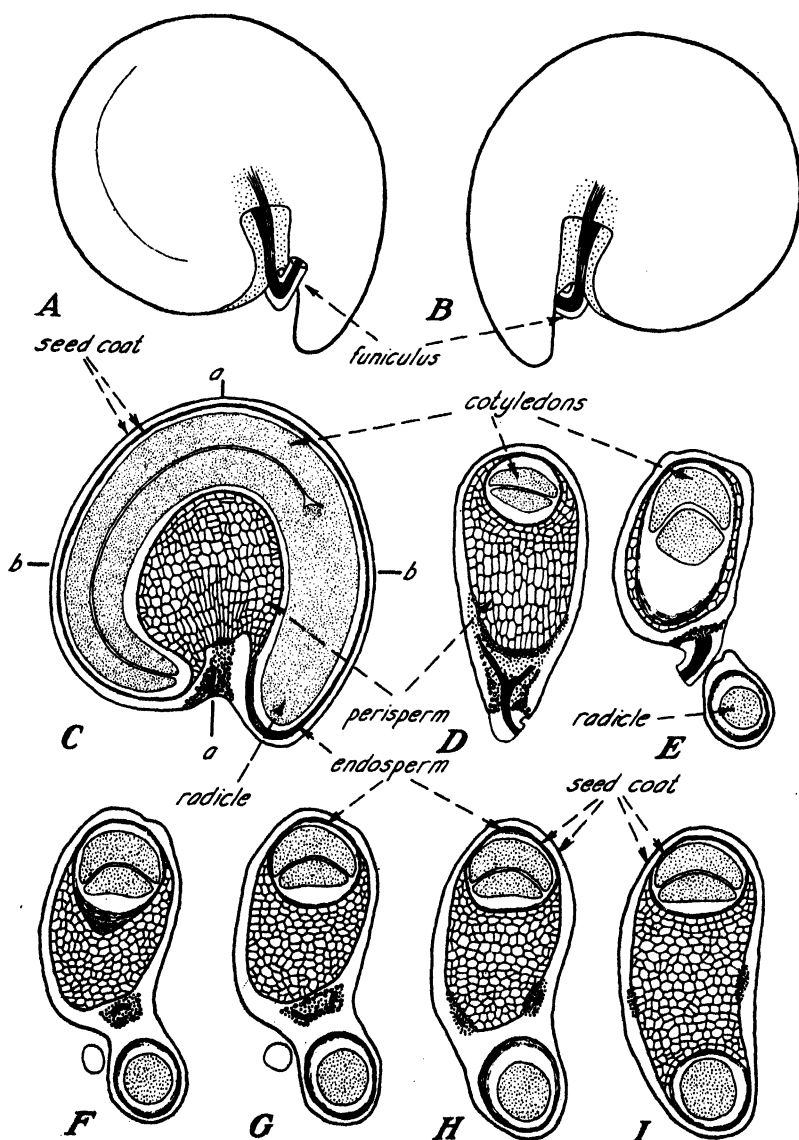
FIGURE 6.—Sugar-beet seed: *A* and *B*, Diagrams showing the distribution of the vascular tissues; *C*, section of mature seed, longitudinal with respect to the embryo; *D*, transverse section made along the axis *a* in *C*; *E–I*, successive transverse sections made parallel to the axis *b* in *C*, *E* being the lowest section. In *C–I*, the xylem is shown in solid black, the phloem by heavy stipples, and tannin-containing cells by circles. All × 18.

germinates. As to the phloem, in the material studied no fully matured sieve tubes were observed in the resting seed, but the young protophloem elements could be readily recognized by the presence of

vacuolating nuclei, sieve-tube plastids, and a coarse network of strands filling the lumina of cells. Sieve-plate perforations were not perceptible.

In partly germinated seed, in which the seed coats were still clinging to the embryo and the perisperm was not yet absorbed, mature sieve tubes were present in the embryo. They were free from nuclei and were "clear" because of the disappearance of the coarse network of strands, and they showed end-wall perforations.
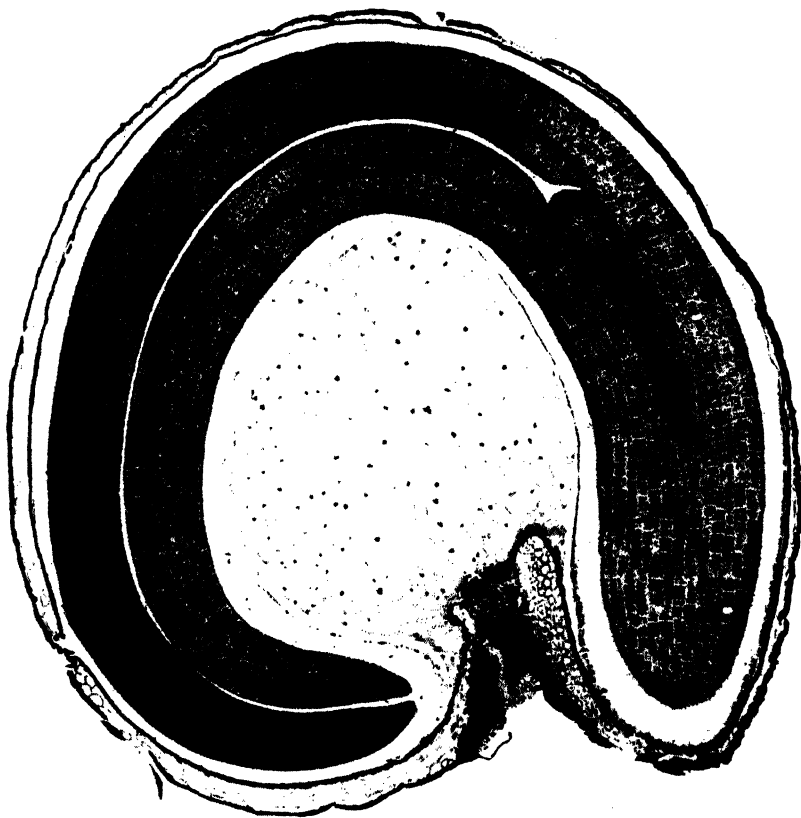
FIGURE 7.—Section through a healthy mature sugar-beet seed, longitudinal with respect to the embryo. The cotyledons are to the left, the hypocotyl to the right. The curved embryo partly encloses the perisperm. The chalaza is below. Compare also with figure 6, *C*. × 46.

Previous studies (*6*, *7*) indicate that curly top virus moves in mature sieve tubes. As the embryo develops such elements before it completes the absorption of perisperm, the virus probably could exist in the embryo if it managed to enter it with the absorbed food.

Studies of virus behavior have given strong evidence that continuity of cytoplasm is necessary to permit movement of virus from cell to cell. No obvious cytoplasmic connection, however, appears to exist between the embryo and the other parts of the seed, unless possibly through the suspensor. In young seeds the latter structure

was readily recognized, but in mature embryos degenerating or empty cells were found in place of the suspensor.

The mature embryo appears to lie free within the embryo sac and its prolongation, the caecum (*1*), as is shown in figure 7. The section in this figure, however, was not cut through the suspensor. It is impossible to obtain a perfect median section of a beet embryo because it is so curved that the tips of the cotyledons and that of the radicle lie in different longitudinal planes.
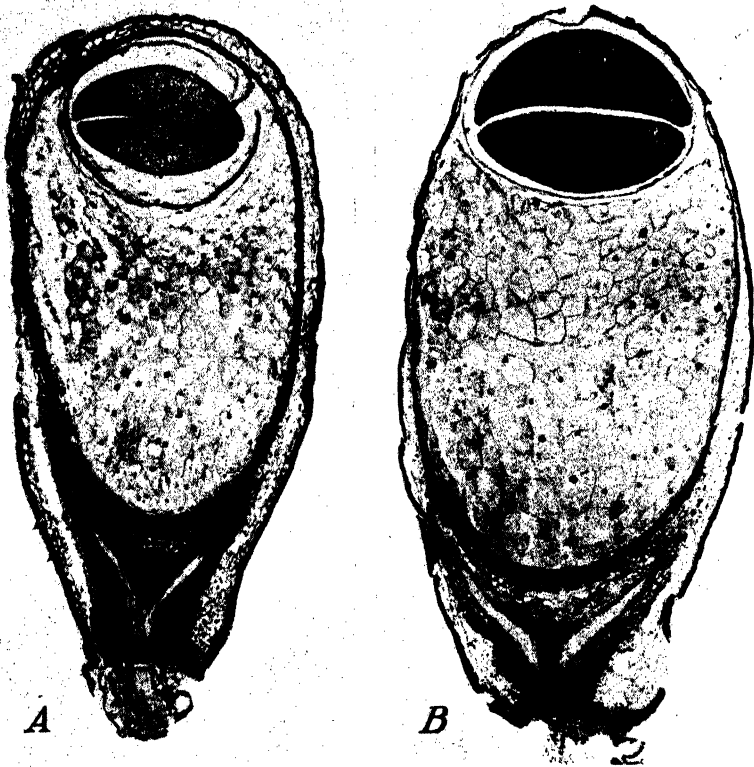
FIGURE 8.—*A*, Section through a healthy sugar-beet seed, transverse with respect to the embryo. Below is a portion of the funiculus, which merges into the chalaza with its forking xylem strand. The perisperm is in the middle, the cotyledons above. × 40. *B*, Section through diseased sugar-beet seed, slightly more mature than the healthy seed in *A*. The phloem appearing above the xylem fork shows hypertrophy. × 40.

One side of the embryo is adjacent to the perisperm; the other side is next to the inner layer of the seed coat. A thin lining of crushed nucellar cells separates the embryo from the perisperm and the inner layer of the seed coat except in the region of the radicle where some endosperm cells occur (fig. 7).

As already mentioned, phloem degeneration occurs in all parts of flowers from affected plants. In the ovule this degeneration becomes evident as soon as phloem develops. As in root tips or young leaves, the development of the first sieve-tube elements in the young floral parts is followed by degeneration of the phloem. Primary hyperplasia, which results in an increased production of sieve tubes, is

very conspicuous in the chalaza so that the phloem in that region is more massive in diseased than in healthy seeds. Degenerating phloem cells occur next to the "passage cells" intervening between the phloem and the perisperm, but the passage cells do not seem to be affected (fig. 8, *B*). No differences were recognized in the structure of embryos and perisperm from healthy and from diseased plants.

In many instances the perisperm cells of healthy and diseased seeds showed shrunken nuclei near the passage region (figs. 7 and 8, *A*) and the cytoplasm formed darkly stained coarse threads on which spherical bodies were strung. Although this fixation image is particularly common in diseased seeds, its occurrence in the healthy seeds seems to indicate that it is not a symptom of curly top.

Tests for Virus

Anatomical observations suggest that the virus invades the vascular area of the seed quite early in its development. To supplement these observations, attempts were made to recover virus from seeds and to determine its location and relative concentration in the different parts. The first tests were made to determine the virus content of the seed as a whole as compared with that of the surrounding pericarp. In these tests mature seeds from green plants were removed from the pericarp, ground in a mortar, mixed with distilled water, and centrifuged. The supernatant liquid was added to an equal volume of 95-percent alcohol, and the resulting precipitate was thrown down by centrifugation. The precipitate was dried, mixed with 1 cc of 5-percent sugar solution, and centrifuged. The supernatant liquid was tested for virus content in the usual way by means of leafhoppers. Seventy-five seeds were used in each test. The pericarps from which the seeds were removed were tested by the same method.

The results of these tests are shown in experiment 2 of table 1. Preparations derived from the pericarp gave considerable infection, but those obtained from the seeds gave a much higher percentage of infection. Mature seeds apparently have a very high concentration of virus.

Location of Virus in the Seed

As illustrated in figure 7, the beet seed is composed largely of perisperm and embryo. By splitting the seed coat at the top of the seed and applying pressure, the embryo may be removed without any apparent injury to it. Soaking the seed in water for a few minutes prior to splitting the seed coat facilitates this operation.

Embryos were removed from seeds from curly top plants and washed several times in distilled water. They were then ground in a mortar, mixed with distilled water, and centrifuged. The supernatant liquid was precipitated with alcohol, and the precipitate was tested for presence of virus as already described. The parts of the seed remaining after the embryo was removed were treated in a similar way, except that in some of these tests 0.5 cc of juice from a healthy beet was added to hasten precipitation with alcohol. The addition of this amount of beet juice apparently had no influence on the amount of virus that was recovered. Seventy-five seeds were used in each test.

The results of these tests are shown in experiment 3 of table 1. Embryos yielded no virus in any one of the eight repetitions of this experiment; whereas preparations from the parts of the seed remaining after the embryo was removed produced a high percentage of infection in seedling beets, showing that the virus in beet seeds is restricted to parts other than the embryo.

After it had been demonstrated that the embryo contains no virus, further tests were carried out to determine whether the virus of the seed occurs in the perisperm or is confined to the small amount of phloem at the basal end of the seed between the seed coat and the perisperm.

In each test 50 mature seeds were used. These were divided into 2 lots of 25 seeds each. One lot was not dissected and served as a control. With the other lot of 25 seeds, an attempt was made to separate the part of the seed containing the vascular elements from a part free from conductive tissue. Two methods were employed to effect this separation. In the first, the chalazal end and two sides of each seed were removed by means of a sharp razor. The parts removed consisted of part of the seed coat, the vascular system, and some of the embryo and perisperm. The remaining part consisted mainly of perisperm and embryo but contained a part of the seed coat. In the second method the seeds were split into two approximately equal upper and lower parts. The lower part contained the vascular elements but the upper one presumably was free from vascular tissue, since the vascular strands extend less than half the length of the seed. In all of these tests the seeds or seed parts were ground in a mortar, mixed with water, and centrifuged, and the supernatant liquid was treated as already described.

The results of these tests are shown in experiments 4 and 5 of table 1. In experiment 4, with seeds dissected by cutting away the parts containing vascular elements, a high concentration of virus is indicated for the seeds as a whole and for the parts containing the vascular tissue. However, considerable virus was obtained also from the parts presumably containing no vascular elements. In experiment 5, where the seeds were cut into two halves, infection was much too low to provide conclusive results.

These latter tests were made in August, during a period of very high temperatures, which probably accounts partly for the low percentage of infection. The results of these two experiments indicate that the place of greatest virus concentration in the seed is the basal end in the region containing the vascular system. They also suggest the probable occurrence of a certain amount of virus in the perisperm, although the possibility of including some vascular tissue with the perisperm in these tests was not eliminated entirely.

Full-size seed coats, apparently fully developed but with no perisperm or embryo, are found frequently in seed balls. A number of such seed coats were tested for virus in the usual way, 25 seed coats being used in each test. As shown in experiment 5 of table 1, no virus was recovered from this material.

Tests were made next to obtain evidence as to whether any virus passes into the embryo or the developing seedling during the process of germination. Mature seeds from infected plants were removed from green seed balls and placed on blotting paper in a moist chamber. The seeds germinated in from 2 to 5 days. When the larger seedlings

had attained a size in which the cotyledons had turned green, the seedlings were tested for virus content separately from the remains of the seed coats and perisperm. Under the conditions of these tests not all of the starch of the perisperm had been utilized by the seedlings. Seeds that did not germinate were discarded.

The results of these tests are given in experiment 6 of table 1. They show conclusively that seeds capable of germinating may contain large quantities of virus and that this virus is unable to enter the embryo or young seedling during the process of germination. There is no evidence of inactivation of virus in the process of germination of the seed. Some of the stored food was utilized and the virus was left behind in the seed coat and attached parts, where it remained in active condition for at least several days.

RELATION OF STAGE OF MATURITY OF SEEDS TO VIRUS CONTENT

The results of previous tests (*2*), in which nonviruliferous leafhoppers were allowed to feed on very young seeds from diseased plants, indicate that the virus is not present in young seeds in quantities sufficient to be detected by the direct feeding method. Since the mature seed has a very high virus content, it seems probable that there is a rapid increase in virus content as the seed matures.

An experiment was made to determine the accuracy of previous determinations of virus content of young seeds and to gain information as to the virus content of seeds in different stages of maturity. Seeds of three ages were used in these tests: (1) Seeds quite immature, seed coats not colored, fertilized eggs in first stages of division, no starch in the perisperm; (2) seeds with light-brown seed coats, embryos about one-third of their ultimate length, endosperm present, perisperm containing some starch; (3) mature seeds, seed coat brown, embryos full length, perisperm well filled with starch. Some of the mature seeds had withered funiculi. The seeds were removed from the pericarp and tested for virus content according to the usual procedure. Seventy-five seeds were used in each determination. The pericarps from which the seeds were removed also were tested.

The results of these tests (table 1, experiment 7) confirm those obtained in other experiments with young seeds and indicate a very low virus content in the seed in the early stages of its development. Evidently, as the seed matures its virus content increases rapidly and reaches a level that apparently is above that of any other part of the plant.

LONGEVITY OF THE VIRUS IN DRY SEEDS

Seeds from three plants affected by curly top were dried at room temperature and tested at intervals of 1 month to determine the length of time the virus remained in active condition. The dry seed balls were soaked in water for several hours before being tested for virus content. Then the seeds were removed from the pericarps, macerated in a mortar, and tested according to the procedure already described. Seventy-five seeds were used in each test.

The results of this experiment, which are given in table 2, show a considerable decrease in virus content in 1 month, a further decrease in 2 months, and apparent absence of active virus in seeds that were stored 3 and 4 months.

TABLE 2.—*Longevity of the curly top virus in dry beet seeds* [1]

Experiment	Plants infected by inoculum prepared from seeds after indicated number of months in storage				
	[2] 0	1	2	3	4
	Number	*Number*	*Number*	*Number*	*Number*
1	15	7	0	0	0
2	13	8	5	0	0
3	16	4	5	0	0

[1] 20 plants were inoculated in each test.
[2] Tests were made before the seeds were dried, as a check.

One of the seedstalks from which seeds were removed was tested for virus content at monthly intervals for 7 months. The results of these tests indicate that the original virus content of the stalk was far below that of the seeds and that active virus content decreased with aging. However, virus was recovered in each of the seven tests, suggesting that virus may be inactivated less rapidly in tissues of the dry seedstalk than in dry seeds.

TESTS FOR SEED TRANSMISSION

Evidence that under ordinary field and greenhouse conditions curly top is not seed-transmitted is rather extensive and quite conclusive. Severin (8) grew 22,738 plants from seeds derived from infected plants and found no case of seed transmission. In the thousands of seedlings grown at the Riverside, Calif., station of the Division of Sugar Plant Investigations, occurrence of disease could in no case be traced to seed transmission, though many of the seeds used undoubtedly came from infected plants. Results of field observations furnish similar evidence.[3]

The observations in the field and in the greenhouse were made, however, on seedlings from seeds that were kept in storage for a time. It has been shown that the curly top virus loses its activity in dried seeds in less than 3 months. Obviously, seeds kept in storage longer than the period during which the virus remains active in stored seeds could produce only healthy seedlings, regardless of their virus content at an earlier date. To eliminate the possibility of inactivation by drying, seeds almost mature but having green pericarps were taken from infected plants and immediately tested for transmission of the virus through the seeds. In some cases entire seed balls were planted in sand. In others, the seeds were removed from the balls and germinated in sand or on blotting paper in moist chambers. The seeds required no rest period and germinated in from 2 to 5 days.

As shown in table 3, germination was relatively high and only healthy seedlings were produced by these seeds. Some of the seeds that failed to germinate on blotting paper were tested for virus content and proved to carry a high concentration of virus. The seed coats and the parts remaining in the seed coat after germination were tested for virus after the seedlings were about 1 inch long, and

[3] Supplemental evidence as to absence of seed transmission is afforded from experiments conducted in Michigan by G. H. Coons and J. E. Kotila, in which sugar-beet seed produced in New Mexico subject to severe exposure to curly top was used in seed-treatment experiments. More than 200,000 plants were concerned in these tests and no case of curly top was found, the plots being critically examined for any such occurrence. (Verbal communication.)

appeared to contain about as much virus as the seeds that did not germinate. These results show that the presence of large quantities of virus in seeds does not necessarily prevent germination, and suggest that the virus content of seeds has no influence on germination. Also, they furnish evidence that the embryo developing into a seedling is able to drain the seed of stored food without acquiring any of the virus that is either stored with the food or present in the immediate vicinity of the stored food.

TABLE 3.—*Results of tests for seed transmission of the curly top virus*

Treatment of seed balls or seeds	Seed balls or seeds planted	Plants produced	
		Healthy	Diseased
	Number	*Number*	*Number*
Seed balls in soil	2, 200	3, 970	0
Seeds in sand	225	141	0
Seeds in moist chamber	150	134	0

STUDIES ON TOBACCO

The symptoms produced on tobacco by curly top are rather characteristic and marked. The first symptoms following infection are very severe. The young leaves show vein clearing, become much distorted, and remain small. The stem also is usually distorted in the region of attachment of first affected leaves. Soon the growing point of the infected plant recovers to a marked degree and frequently shows only translucent veins in the very young leaves in subsequent growth. Such partial recoveries are due to plant responses and not to attenuation of the virus. Although vein swelling is less marked in tobacco than in beet, usually it is perceptible on the calyx and fruits of affected plants. Surface exudate from the phloem, which is common in the beet, has not been observed in tobacco.

The tobacco plant was selected for a study of the relation of the curly top virus to different tissues because some information on the movement of virus was already available for this plant from previous ringing experiments (*2*). These indicate that the virus will not pass through the woody cylinder. Moreover, this plant is different from the beet in anatomical structure. The presence of internal phloem and absence of anomalous growth affords an opportunity for making determinations of virus content of two distinct phloem-containing regions as well as of woody tissue and pith. Also, its flowers are large enough for determination of virus content of the various parts. This is especially important in the determination of virus content of different regions of the anthers. Stamens of beet are too small for satisfactory use in such a study.

Since the liquid content of the phloem does not move to the surface of leaves and petioles in diseased tobacco plants, it seems probable that movement of phloem content into the intercellular spaces of the cells of tissues adjacent to the vascular bundles may not occur or may be less extensive than in beet. If no phloem exudate occurs in the intercellular spaces of parenchyma of tobacco, the amount of virus recovered from the juice of such tissue should indicate to some extent the virus content of the parenchyma cells themselves.

VIRUS CONTENT OF VEGETATIVE PARTS

Phloem degeneration in Turkish tobacco plants affected with curly top passes through stages similar to those observed in the sugar beet affected by the same disease (6), but the necrotic tissue shows less pronounced accumulations of darkly staining material (cf. figs. 1; 9, *B*). In the final stages of degeneration the collapse of cells leads to a formation of internal cavities that may be seen in freehand sections as well as in paraffin preparations. Figure 9, *A*, shows a leaf vein from healthy tobacco and figure 9, *B*, shows a vein from a comparable leaf affected by curly top. The collapse of cells in the diseased leaf is perceptible in both the external and the internal phloem. The xylem is slightly hypoplastic.

Leaves and different parts of the stem were tested for their virus content. Plants used for these studies had been inoculated by the grafting method and were allowed to grow until they were almost mature. Except in two tests, leaves and stems selected for the test were produced after the plants were infected. Entire leaves were used, and the stems were dissected into (1) bark,[4] (2) wood, (3) internal phloem including considerable pith, and (4) pith. Tests were made from preparations from expressed juice as already described.

The results of these tests are shown in experiment 1 of table 4. They indicate that juice derived from the bark and from the internal phloem had a relatively high virus content, whereas that derived from the wood and pith had a rather low virus content. These results are similar to those obtained from the vegetative parts of beets (table 1, experiment 1), although the percentage of infection from wood and pith of tobacco was somewhat higher than that from parenchymatous tissue of beet.

TABLE 4.—*Virus content of different organs and tissues of tobacco plants*

Experiment no.	Source of virus	Tests [1]	Tests that yielded virus	Plants inoculated	Plants infected	
		Number	*Number*	*Number*	*Number*	*Percent*
1	Leaf	6	6	120	35	29.1
	Bark	9	8	180	41	22.7
	Wood	9	3	180	7	3.8
	Internal phloem	9	6	180	56	31.1
	Pith	9	3	180	8	4.4
2	Calyx	3	2	60	26	43.3
	Corolla	3	2	60	18	30.0
	Filaments	3	3	60	13	21.6
	Anthers	2	2	40	12	30.0
	Pistils	4	1	80	1	1.3
3	Pollen	5	0	100	0	0
	Anther (remains)	2	2	40	12	30.0
4	Parenchyma of anthers	9	0	180	0	0
	Phloem of anthers	9	3	180	5	2.7
	Anthers	4	2	80	4	5.0
5	Capsule wall	2	2	40	4	10.0
	Placenta	3	3	60	22	36.6
	Seeds	6	4	120	8	6.6

[1] 20 plants were inoculated in each test.

[4] The term "bark" as used in this paper signifies all tissues of the stem from the epidermis to the cambium, inclusive.
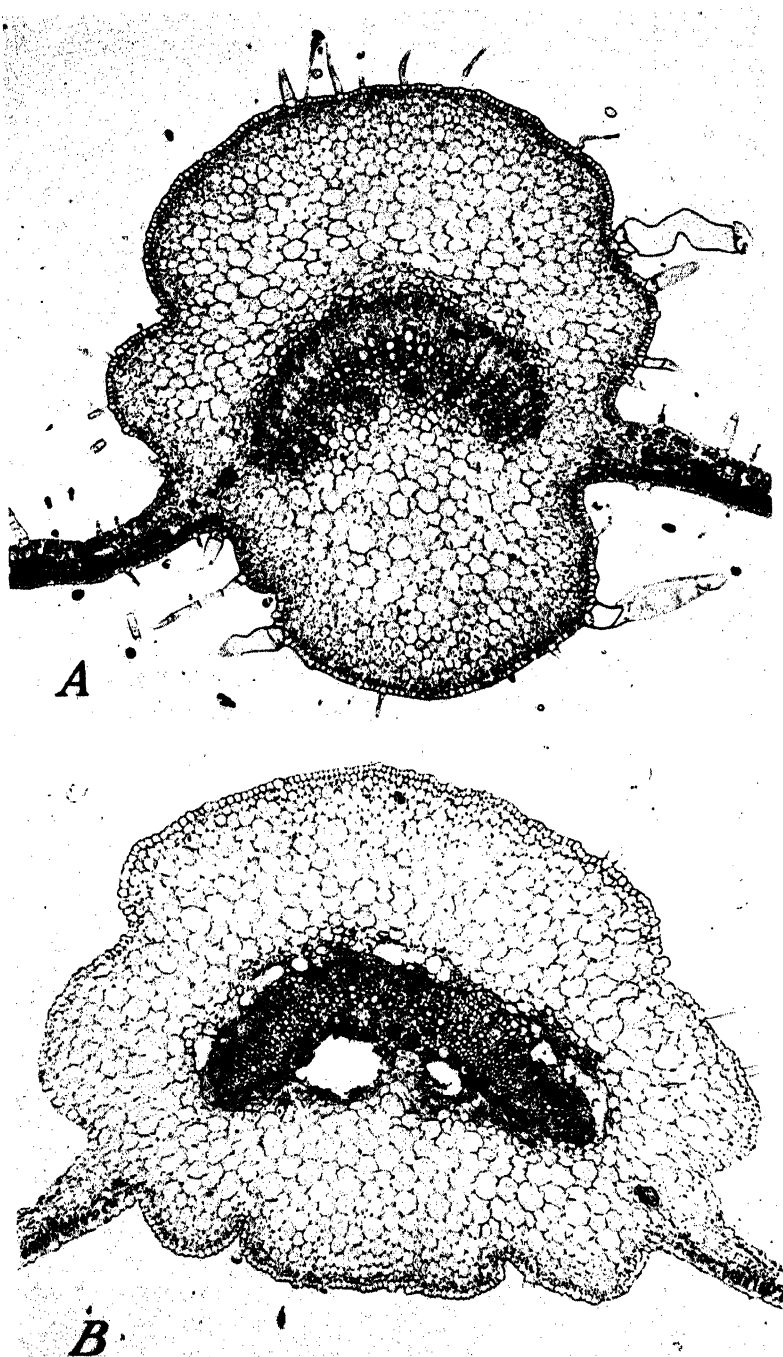
FIGURE 9.—Transverse sections of midribs of leaves of *Nicotiana tabacum: A*, From a healthy leaf; *B*, from a diseased leaf. The phloem in *B* is in advanced stage of degeneration; it shows collapse of cells but no phloem exudate. × 41.

VIRUS CONTENT OF FLOWER AND FRUIT PARTS

The calyx, corolla, pistil, and stamens of the tobacco flower show distinct vein swelling and a certain amount of distortion, even in plants that otherwise appear healthy. Phloem degeneration is evident in all flower parts of diseased plants, and occurs even in the smallest bundles in which only a few sieve tubes are present.

VIRUS CONTENT OF FLOWER PARTS

The relative virus content of various parts of the flower was determined in flowers from plants that had been diseased more than 2 months. The flowers were dissected into calyx, corolla, anthers, filaments, and pistils. The tests of these organs were made in the usual way on preparations from expressed juice. The results given in experiment 2 of table 4 show that virus occurs in all of these structures, although relatively little was recovered from pistils.

FURTHER TESTS OF ANTHERS

Additional tests were made to determine in what part of the anther the virus is located, and, particularly, whether it occurs in the pollen. The tobacco anther contains considerable pollen and also furnishes rather favorable material from which to obtain tissue free of phloem elements. The vascular strand passing through the filament into the connective does not ramify but continues as a single strand almost to the apex. It is rather narrow in the filament, but widens out in the lower and middle parts of the connective, again becoming very narrow toward the apex of the anther. It is amphicribral in structure, the narrow xylem strand being surrounded by phloem.

Figure 10, *A* to *G*, represents transverse sections through the mature anther; whereas, figure 10, *H*, shows a whole anther seen from the dorsal side. The anther is divided longitudinally into a right and left lobe, each with two locules. At maturity, the connective tissue, which joins the two lobes and forms a partition between the two locules, separates from the side walls of the anther, as shown in *B* to *F* of figure 10. Each of the side walls bears a longitudinal line of dehiscence, indicated in the figure by very dense stippling.

Although the vascular and the sporogenous tissue of the anther are not in contact with each other, there are some thin-walled parenchyma cells that appear to serve as a connection between the two. These cells are represented by rather heavy stippling in *A* to *G* of figure 10. They make up the partition between the pollen sacs and form a narrow layer between this partition and the vascular strand. Similar thin-walled cells occur around the vascular strand also, but in figure 10 these cells are included with the vascular tissue in the cross-hatched circle. In young anthers these parenchyma cells are like the other sterile cells making up the anther walls, but in mature anthers they differ strikingly from the adjacent cells; they remain thin-walled, while the other cells of the anther walls develop the characteristic secondary thickening. The appearance and distribution of the thin-walled cells suggest that these cells are the tissue that conveys the materials from the phloem to the developing pollen.

Tests were carried out to determine whether virus passes out of the phloem of the connective into the surrounding tissue of the anther, especially into the pollen. About 0.1 g of pollen, which was

shaken from mature anthers, was used in each test. The anthers from which the pollen was taken were also tested for virus. The material was ground in a mortar, mixed with water, and centrifuged, and the supernatant liquid was tested as in the case of the expressed juice.
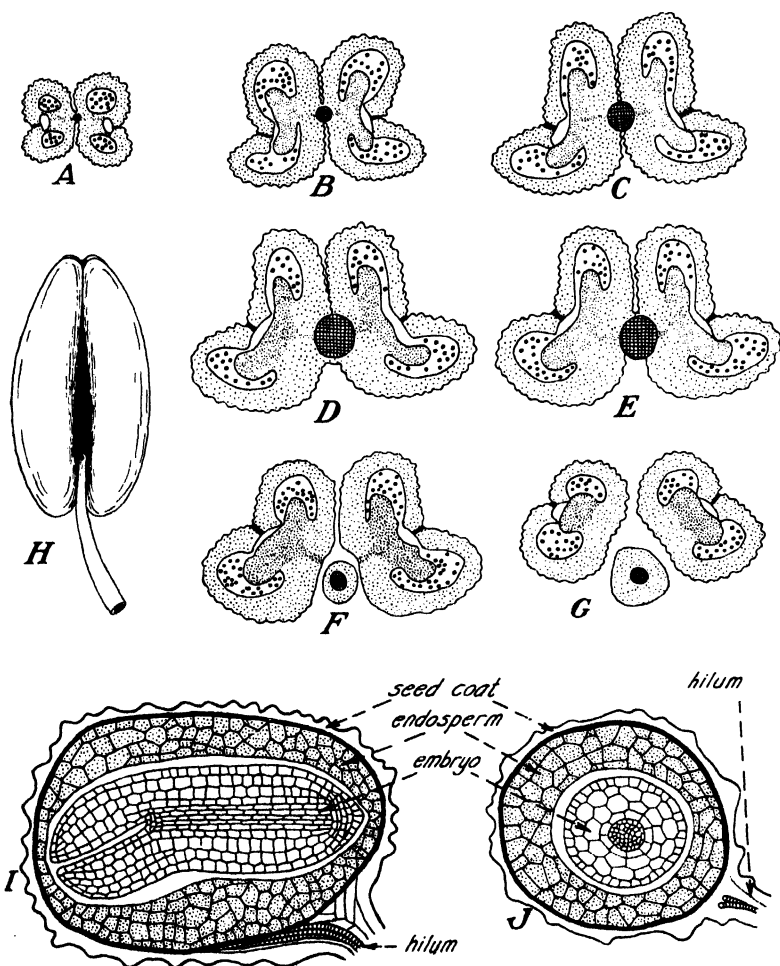
FIGURE 10.—*A-G*, Transverse sections made at different levels through an anther of healthy *Nicotiana tabacum*, (*A*) showing the upper, (*G*) the lower part of the anther. The cross-hatched circles indicate the position of the vascular tissue. × 22. *H*, General view of a tobacco anther seen from the dorsal side where the filament is attached. × 11. *I*, Longitudinal, and *J*, transverse section through a mature seed from a healthy plant of *Nicotiana tabacum*. × 71.

The results of these tests are given in experiment 3 of table 4. They show that virus was not recovered from the pollen, but was obtained from the anthers from which the pollen was removed.

Further efforts were made to recover virus from parts of the anther containing no phloem. Anthers not yet open were split longitudinally through the two halves, thus separating the outer tissue containing no vascular tissue from the part containing the

connective. In each test, 100 anthers were separated in this manner and the two parts were tested for virus as in the previous experiment. One hundred whole anthers were used as a check in each test.

The results of these tests are shown in experiment 4 of table 4. No virus was obtained from tissue devoid of vascular strands. However, the virus content of the part containing vascular tissue was apparently very low, as was that of the check. Possibly, young anthers contain less virus than more mature ones.

SEEDS AND CAPSULES

As stated previously, in capsules of diseased plants the veins are distinctly swollen and the phloem is necrotic, furnishing evidence of the presence of virus. However, the seeds develop and mature normally so far as determined, though their number may be reduced in some cases.

The tobacco seed contains a straight or a slightly curved embryo which is surrounded by endosperm (fig. 10, *I*). The embryo and endosperm do not appear to be connected with each other. Their cells are packed with nitrogenous storage material. No suspensor was observed in the mature embryo. The hilum, a scar indicating the place of attachment of the funiculus to the ovule, forms a projection on the surface of the seed (fig. 10, *I*). The vascular tissue passes without branching through the hilum region and for a short distance through the parenchyma between the outer and inner layers of the seed coat. The position of the vascular strand is indicated by the raphe on the surface of the seed.

Tobacco seeds are much too small to be dissected for determinations of virus content of their various parts, but tests were made of mature seeds, capsule walls, and placenta. One-half gram of material was used in each test, and the procedure followed was the same as that with pollen.

The results of these tests are shown in experiment 5 of table 4. Virus was obtained from all parts of the capsule, including the seed. The results suggest that the virus concentration in tobacco seeds may be much less than that in beet seeds.

DISCUSSION

The results of experiments reported in this paper show conclusively that in some instances the parenchyma tissue selected from the crown and flowering stalk of beet and from the pith of tobacco stems contained virus. However, in every case the virus content was far below that of adjacent regions in which vascular bundles were present. The exact location of the virus in respect to the parenchyma cells is difficult to determine, since there are at least four possible relations that must be considered and evaluated before definite conclusions can be reached. It is possible that the virus may have been present (1) in undetected vascular strands, (2) in surface contaminants from the phloem of adjacent tissues, (3) in phloem exudate in the intercellular spaces, and (4) in the parenchyma cells themselves.

Since no vascular strands were found in any of the parenchymatous regions examined microscopically, it seems probable that vascular tissue was not an important source of virus in these tests. Also, contaminants probably contributed very little to the total amount

of virus recovered from parenchyma, since the washing to which the carefully dissected sections were subjected would be expected to reduce surface contaminants to such an extent that virus in portions not removed would be difficult to detect by the methods employed. It seems probable, therefore, that at least the larger part of the virus recovered from parenchyma of beet and tobacco was derived either from phloem exudate in the intercellular spaces or from the cell themselves.

Although a special effort was made to select plants that showed no leakage of phloem content into the tissues adjacent to the phloem, obviously it is impossible to determine definitely that no such leakage occurred. For the most part, the parenchymatous regions selected for tests were developed in diseased plants several weeks before they were tested for virus content. It is possible that there may have been leakage of phloem content into the intercellular spaces, especially in the earlier stages of development of the tissue, that could not be detected. The resistance of the virus to inactivation by influences likely to be encountered in intercellular spaces is probably sufficiently high to enable it to persist in active condition in such an environment for several weeks.

Since the virus content of the intercellular spaces cannot be determined by direct methods, the evidence on the question of the relation of virus to parenchyma must of necessity be indirect. Failure of virus to traverse medullary rays in tobacco (*2*) indicates inability to move through at least some types of parenchyma. However, the apparent recovery of virus from the perisperm of the beet seed, a tissue in which there are no intercellular spaces, and the recovery of virus from parenchyma of resistant beets and from parenchyma of tobacco point to the probable occurrence of small amounts of virus in certain types of parenchyma.

Perhaps, on the basis of plant structure, it should be expected that at least a small amount of virus occurs in the parenchyma in regions adjacent to the phloem. The presence of a high concentration of virus in the phloem has been definitely established. If protoplasm of the phloem is continuous with that of surrounding cells through the plasmodesma, probably a certain amount of virus would diffuse through the plasmodesma from the phloem to the adjacent parenchyma cells, the diffusion being, perhaps, accelerated by protoplasmic streaming. The magnitude of such an invasion would depend on the rate of outward movement. The virus content, however, would be determined not only by this rate but also by the ability of the virus to multiply in parenchyma and by the rate of inactivation of the virus by parenchyma. Even if it is assumed that all or a large share of the virus recovered from parenchyma is of intercellular origin, the low concentration indicates that the virus either has difficulty in entering parenchyma cells or is inactivated in a short time after coming in contact with the protoplasm of such cells.

The presence of large quantities of virus in the seed of beet and tobacco affords a basis for further speculation regarding the relation of virus to certain types of tissues. Previously (*2*) it was suggested that a tissue barrier exists between the phloem of the mother plant and the young sporophyte. In the beet seed, virus is probably more concentrated in the vicinity of the vascular elements, but it is by no means evident that it is confined to these elements. In fact, the

evidence seems to indicate that some of the virus escapes into regions outside the phloem and perhaps occurs in the perisperm itself. A mechanism that permits an embryo to develop to its full size in the seed and to germinate later, using up a part or all of the food stored in the surrounding tissues, and to remain free from virus, offers indeed an effective barrier to virus movement. No obvious morphologic structure that would account for this phenomenon was detected. The layer of compact tannin-filled cells separating the phloem from the perisperm might be suspected to function in restricting virus to the vascular region. Evidence, however, points to the occurrence of virus on both sides of this layer and suggests that even if virus were able to pass through the tissues surrounding the embryo, including the perisperm, it might still be unable to enter the embryo. Since the embryo develops from a single fertilized cell and grows by absorbing neighboring cells, it may have no protoplasmic connections with adjacent cells, except possibly in the suspensor. If there is no protoplasmic connection, virus would be required to pass out of the adjacent cells through the surface cells of the embryo in order to infect the embryo. In view of the considerable evidence that cell walls, especially those of the epidermis, tracheae, and root hairs, are not permeable to the virus, there seems good reason to suspect that lack of protoplasmic union between the embryo and adjoining tissues would effectively prevent passage of virus into the embryo, even though the perisperm might be thoroughly saturated with virus.

The mechanism that protects from infection the male and female gametophyte and the embryo in its initial development is of considerable interest. Difficulty of invading meristem and parenchyma probably would afford protection to these structures from the curly top virus but additional factors must be operative with such viruses as that of tobacco mosaic, which is undoubtedly able to invade extensively the common types of parenchyma.

If the results of this study indicate relative virus concentration in different parts of the beet plant, the concentration of virus is greater in the seed than in any other part of the plant. Concentrations of virus in the seed greater than those in other parts of the plant could be reached only by multiplication of the virus in the seed or by accumulation against a virus gradient. It is possible that the conditions for multiplication of the virus are very favorable in the developing seed and that the high virus content is produced by multiplication. However, there is no evidence to support this view.

Apparently the increase in virus content of the seed parallels, more or less, the increase of food reserves of the seed. It may be that virus movement is associated in some way with food translocation and that the high virus content of the seed is the result of movement of virus into it from other parts of the plant. In this connection it may be pointed out that mass movement of food materials in the phloem, as suggested by Crafts (4) might result in a high virus content in the seed even though no multiplication occurred there. Since virus is present in the phloem it seems reasonable to assume that in a mass movement of liquid content of the phloem into the seed the virus would be carried in also. Of the materials transported to the seed by mass flow, the water would be transpired or utilized, the mineral elements would be utilized or stored, and the sugars would be stored

as starch. Under these conditions, virus would accumulate as a residue and probably would reach a high concentration.

SUMMARY AND CONCLUSIONS

Anatomical evidence indicates that the curly top virus invades the phloem of the entire vascular systems of beet and tobacco. In susceptible varieties of beet the disease is characterized by necrosis of the phloem and hypertrophy and hyperplasia of the phloem and pericycle. Liquid content of the phloem moves through the intercellular spaces of the extraphloem tissue and accumulates on the surface of petioles and leaves.

In plants of the resistant beet variety U. S. 33, anatomical abnormalities in the phloem are less extensive and, so far as observed, very little exudation from the phloem occurs. No anatomical abnormalities were observed in the parenchyma of the ventral side of the petioles or in the pith of the crown and flowering stalk in this variety.

Phloem degeneration in diseased tobacco plants passes through stages similar to those observed in beet. However, the necrotic areas become cavities and are not filled by proliferation of cells adjacent to the necrotic regions, as in beet. No phloem exudate was observed in the intercellular spaces of tissues outside of the phloem, and phloem exudate did not occur on the surface of leaves and petioles. Transverse sections of stems of plants used in these studies appeared normal except for areas of degeneration in the external and internal phloem.

Tests of different tissues of resistant beets indicate a very low virus content in parenchymatous regions of the crown and stalk and in the ventral side of petioles, as compared with adjacent regions containing vascular bundles. Also, much less virus was obtained from wood and pith of tobacco than from bark and from tissue containing internal phloem.

Immature beet seeds have a very low virus content. This content increases as the seed matures, and a very high virus concentration is reached in the mature seed. Apparently this virus is most abundant in the vascular region of the seed, but it is probably not restricted to the phloem. No virus was recovered from the embryo. Seeds heavily charged with virus germinated readily and the developing embryos utilized the food stored in the perisperm without becoming infected. No case of curly top developed in 4,245 plants grown from seed balls produced on sugar beets affected with curly top, tests being conducted before inactivation of the virus in the seed occurred. The virus was inactivated in dry seeds in 3 months.

Virus was recovered from the seeds, capsule wall, and placenta and from all of the flower parts of tobacco. No virus was recovered from pollen or from parts of the anther containing no vascular bundles.

The evidence obtained in this investigation supports the concept that the curly top virus is intimately associated with the phloem of infected plants. The phloem is probably the tissue in which the virus multiplies and it is evidently the tissue in which the virus attains its highest concentration. The exact relation of the virus to tissues other than phloem is uncertain, since it was not possible to determine whether the small amount of virus recovered from the extraphloem regions was derived from the cells themselves or from other sources. However, if virus occurs in parenchyma cells its concentration in such

cells is evidently very low and the conditions are probably very unfavorable to multiplication and spread.

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A MOSAIC DISEASE OF IRIS [1]

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INTRODUCTION

A mosaic disease of bulbous iris was prevalent in California, Oregon, and Washington in the earlier years of commercial plantings in these States. Surveys made during 1928 and 1929 failed to reveal in these States a single large planting that was entirely free from the disease. The percentage of mosaic in original importations from the Netherlands varied widely (from 2 to 90 percent), but there was strong field evidence of spread of the disease after the arrival of the irises in this country, notably in southern California. Some varieties in the older established plantings approached 100 percent infection when the mosaic first came to the attention of the writers. The early presence of the disease in the Netherlands is attested to by Atanasoff.[2]

Preliminary efforts to transmit the disease in 1928–29 were not successful and are not here detailed. The first extensive trials were made in 1929. The senior author left the work in the fall of 1929, the junior author taking it over in the winter of 1929–30 and continuing to the present time. The purpose of this paper is to present the general aspects of the problem, especially the results of studies on the symptoms and transmission of the disease. A preliminary notice [3] of the disease has already appeared.

SYMPTOMS

SYMPTOMS IN BULBOUS IRISES

The symptoms of mosaic in bulbous irises include general dwarfing of the plant, mottling of the leaves, and "breaking" of the flowers. The commercial importance of the disease lies chiefly in the dwarfing effect, which results in a shorter flower stalk for cut blooms and in a lower rate of increase in planting stock. The quality of the flower is also inferior in diseased plants, and the cut blooms do not last as well as normal flowers. Under forcing conditions irises affected with mosaic are conspicuously inferior; in some of the less tolerant varieties they are worthless.

When affected plants are in bud or flower, mosaic mottling of light-green and bluish shades is readily seen and is especially prominent on the leaf bases and bud sheaths. The bud sheath is typically marked with bluish-green blotches on a pale-green ground, or less commonly with yellowish streaks (fig. 1). On immature plants, especially during the early winter growth, mosaic appears as a yellow

FIGURE 1.—Mosaic mottling in a bud of Imperator iris.

streaking of the leaves. Yellow streaking due to cold injury or to bulb rots also appears at this stage, and diagnosis of mosaic is very uncertain until mottling shows in the new growth. Leaf symptoms are similar in varieties of bulbous irises of all classes, including all flower colors, but the flower symptoms vary. In plants affected with mosaic the younger leaves and bud sheaths are always more plainly mottled than the older. The typical mottling of young leaves (fig. 2, *C*) serves to distinguish the symptoms of mosaic from a distinctive whitish square pattern (fig. 2, *D*) appearing in older leaves of varieties subject to winter injury. This winter-injury pattern is not transmissible. Typical mosaic mottling can be produced on winter-injured plants by inoculating the virus into a variety subject to the latter type of injury.

Flower breaks are usually darker than the normal color of the flower. For example, broken flowers of blue Imperator develop nearly black marks on a blue ground; those of .white D. Haring show a purple mottling on normal white falls; and those of lavender Therese Schwartze exhibit prominent purple blotches. The most characteristic flower marking consists of a series of dark blotches of teardrop design (fig. 2, *A*), the tail of the drop extending toward the throat of the flower. Feather-like cleared streaks, lighter than the normal flower color, are associated with mosaic in yellow varieties. The occasional ribbon-like striping of flower parts does not seem to be associated with a virus. This pattern occurs in both healthy and diseased plants and is not transmitted with the mosaic when inoculations are made from plants showing both mosaic symptoms and the striping pattern.

Variations in flower reaction are often observed in a group of individuals of a given variety inoculated with mosaic. Flowers on some plants may be typically blotched on both standards and falls, while those on other plants show few or no markings. An example of this variation in flower response to mosaic inoculation is shown in table 1. The following record may be cited as a further example: Sixteen plants

FIGURE 2.—Symptoms of mosaic in bulbous iris: *A*, Diseased flower of Therese Schwartze showing characteristic "teardrop" markings; *B*, normal flower of Therese Schwartze; *C*, yellow-green stripe mottling characteristic of mosaic in young leaves of David Bles; *D*, white and green angular mottling caused by winter injury in Rembrandt (contrasted with *C*).

of the Therese Schwartze variety that exhibited mottled leaves and mottled flowers in 1931 were dug, divided into planting units of one to four bulbs each, replanted, and left in place for 3 years. This subdivision involved 39 clumps. Of these, 19 bore only mottled flowers every year; 12 bore normal (unmottled) flowers in some years; and 8 bore some normal and some mottled flowers on the same clump in some years. There was no apparent correlation between yearly climatic variations and flower symptoms. Successful subinoculations have been made from plants showing typical leaf mottling but no flower symptoms, as well as from plants exhibiting both leaf and flower patterns. This suggests that more than one virus may be involved in the disease herein called iris mosaic, but the evidence is not yet adequate to permit a definite conclusion on this point. In general the symptoms of inoculated plants agree with those of the source plant, severe mosaic resulting from severe and mild mosaic from mild.

TABLE 1.—*Effect of different symptom complexes on height attained by the Dutch iris Therese Schwartze in 1934* [1]

Type symptoms	Type symbol	Cases developed	Mean height of plants and probable error
		Number	*Cm*
Leaves streaked; flowers strongly mottled, with elliptical breaks	1S	29	38. 8±0. 88
Leaves streaked; flowers average mottled, with elliptical breaks	1A	22	51. 9±1. 40
Leaves streaked; flowers mildly mottled, with elliptical breaks	1T	5	56. 2
Leaves streaked; flowers strongly darkened, with ribbonlike stripes	2	1	70. 0
Leaves streaked; flowers apparently normal	3	3	59. 0
Healthy iris, inoculated but not infected	H	46	68. 6± . 57

[1] Uniform stock inoculated by hypodermic needle with standard Therese Schwartze mosaic inoculum in 1933.

The symptom most consistently associated with mosaic is the tendency to a reduction in size and length of shoots. Table 1 furnishes examples of the dwarfing effect associated with mosaic symptoms of different degrees of severity in a uniform lot of Dutch irises. Uniform round bulbs of healthy Therese Schwartze were selected and planted in the fall of 1932. In the spring of 1933 the resulting plants were inoculated by the hypodermic-needle method with uniform composite juice inoculum from a number of mosaic-affected Therese Schwartze plants. The inoculated plants were left undug in the field, and the reaction and measurements of height shown in table 1 were recorded in the progeny in the spring of 1934. The entire block of 106 plants was inoculated, the healthy plants listed in the table representing the individuals that escaped infection.

Analysis shows that plants of the strongly mottled type 1S and of the average mottled type 1A are significantly shorter than those of the healthy class, and that type 1S is shorter than type 1A. No conclusions can be drawn in regard to the other symptom complexes mentioned in table 1, because of the small number of these types (1T, 2, and 3) that appear in the experiment.

SYMPTOMS IN NONBULBOUS IRISES

The symptoms of mosaic in nonbulbous irises are essentially the same as those described for bulbous irises. The thinness of the blade-like leaves of garden forms renders the pale areas more evident, especially by transmitted light.

Many of the dwarf forms of bearded irises show a general mottling that does not exhibit cleared areas. Transmission tests with these are still in progress, but the evidence thus far obtained suggests that not all such mottling is of virus origin. The symptoms in William Mohr, reputed to be a cross between white-bearded Parisiana and *Iris gatesii* Foster, a giant-flowered member of the Oncocyclus section, are of particular interest because of the popularity and high price of this variety. All representatives of William Mohr that the writers have seen are stunted and during the period of active growth are strongly mottled. Yellow, somewhat diffuse, chlorotic areas are developed in some individuals but not in all. Inoculations from this variety to bulbous irises produced a mild mottling but one definitely related to the virus disease here discussed.

CELLULAR CHANGES

There are few morphological features that seem to be related to the activities of the mosaic virus within the tissues of the types of irises studied. The cell arrangement in a section of iris leaf affected with mosaic agrees perfectly with that in a similar section from a healthy leaf. Two gross tissue changes usually accompany external symptom expression: (1) Reduction in number of chlorenchymatous cells and mature chloroplasts in areas beneath the epidermis where extreme mottling is visible, and (2) reduction in size of the epidermal cells of leaves that have been materially shortened by mosaic infection. The reduction in number of chlorenchyma cells and chloroplasts is not always apparent and is unreliable as a criterion of virus infection. The reduction in size of epidermal cells is surprisingly constant and is easily demonstrable by photographing at the same magnification epidermal peelings from healthy and from diseased plants, as shown in figure 3.

Dufrenoy [4] used mosaic-affected iris material in reaching his conclusions and generalizations relative to the changes induced in cells in response to virus infection. A study of several varieties of bulbous irises and of a few nonbulbous forms indicates that the cellular details vary greatly with the variety, the age of the leaf, and possibly with seasonal changes. The vacuolation processes and differentiation of visible byproducts, including X-bodies, do not keep pace with the production of prominent external symptoms. For example, sections of obviously diseased Imperator leaves collected in December 1934 and of young and old leaves of mosaic-affected Therese Schwartze collected in December 1934 and January 1935 showed agglutination of foreign material and strong vacuolation in only one of many leaves examined. In May 1935, during the flowering season, a number of varieties were studied by fixing leaf epidermal strips in acetoformalin, staining in light green, and passing them through dioxan into Canada balsam. In such mounts the epidermal cells are not broken, and their

⁴ DUFRENOY, J., and DUFRENOY, M. L. CYTOLOGY OF PLANT TISSUES AFFECTED BY VIROSES. Phytopathology 24: 599–619, illus. 1934.

contents remain in place. The writers have repeatedly checked this technique with living material and find that it leaves visible cell entities produced through virus action in a condition directly com-
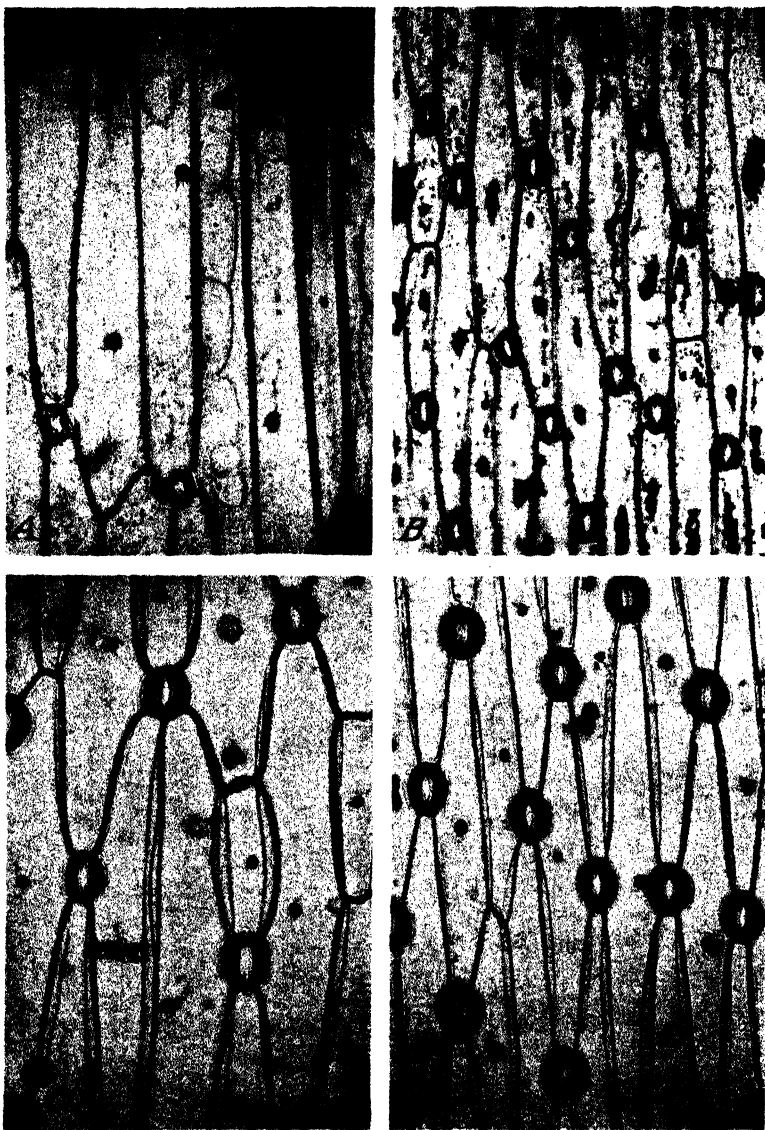
FIGURE 3.—Reduction in size of epidermal cells in iris affected with mosaic: *A*, Healthy Hart Nibbrig; *B*, diseased Hart Nibbrig. Note smaller cells and increased number of visible byproducts in diseased tissue. *C*, Healthy *Iris ricardi; D*, diseased *I. ricardi.* Note smaller cells in diseased tissue. × 150.

parable with but far more readily discernible than those in living material mounted in isotonic solutions. With this method it was found that at blooming time the epidermal cells of mosaic-diseased

individuals of some varieties are filled with visible byproducts, while comparable epidermal cells of other varieties remain indistinguishable from healthy ones. Hart Nibbrig in particular is a good variety for study, since it regularly assumes the virus mode of cell disfiguration (fig. 4).

Iris ricardi Hort. during late spring bore strongly mottled leaves when diseased, but the epidermal cells contained normally hyaline protoplasts. Six months later the cell content of aged foliage exhibited more visible byproducts and, in a few instances, partly organized X-bodies. Even in Hart Nibbrig there is little uniformity in the content of morbid cells of comparable leaves from different individuals; there is also some variation, not attributable to age, in similarly located cells of different leaves of the same plant.

The ultimate cellular inclusions, known as X-bodies, are usually not well formed in irises. The vacuolate type is sometimes formed
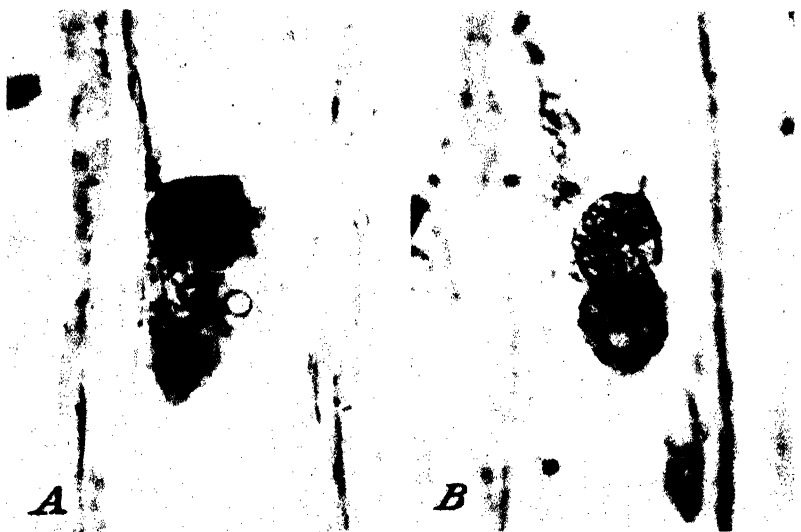
FIGURE 4.—Two types of X-bodies observed in iris affected with mosaic. *A*, Reticulate type in *Iris ricardi*; *B*, Vacuolate type in Hart Nibbrig. Nucleus above in each case. × 1,400.

in epidermal cells of Hart Nibbrig. With a 1.40 N. A. lens and critical illumination these vacuolate types may be resolved into short line-like components. These lines seem to represent the edges of almost ultramicroscopic crystals concentrically arranged around the vacuoles (fig. 4, *B*). The X-bodies within iris cells usually consist of agglutinations of elongate particles which Dufrenoy considers of mitochondrial origin. They are found in cells where the vacuolate type of X-bodies is uniformly absent (fig. 4, *A*).

The epidermal cells of flecked areas in mosaic-diseased flowers contain fewer plastids than corresponding areas of healthy flowers. Comparable areas of diseased and healthy flowers of Therese Schwartze were found to contain 59 and 169 plastids, respectively. This difference is far more noticeable than the similar difference that may obtain in chlorenchyma cells.

Examination of the epidermal cells of the rectangular mottled areas resulting from winter injury in the Rembrandt variety (fig. 2, *D*) reveals more visible particles than the average iris epidermis. The nuclei of these cells are very large, elongate, frequently dumbbell-shaped, and surrounded on all sides by agglutinations of particles. The virus-diseased cell picture differs from this in that the nuclei appear normal and the agglutinated material is adnate to one end or side of the nucleus or present in some other part of the cell.

In the opinion of the writers, internal cell differentiation would be an unreliable criterion for the detection of mosaic in irises. When such differentiation occurs it may be prominent, as Dufrenoy maintains, but it is of quite different form from that induced by fungi that attack iris leaves.

TRANSMISSION TESTS

MECHANICAL METHODS

The mechanical methods used in the experimental transmission of mosaic and the results obtained during five growing seasons are shown in table 2.

TABLE 2.—*Mechanical transmission of iris mosaic*

Season	Method	Total trials	Positive trials	Plants inoculated	Plants infected	
		Number	*Number*	*Number*	*Number*	*Percent*
1929–30	Wedge insertion	38	29	310	103	33
1930–31	do	12	8	74	48	65
	Hypodermic needle	3	1	5	4	80
	Wedge insertion	1	1	11	10	91
	Hypodermic into nodes	34	19	449	60	13
	Hypodermic into stems	1	0	13	0	0
	Hypodermic into leaves	2	0	23	0	0
1931–32	Hypodermic into bulbs	1	0	17	C	0
	Cutting flower stalks	2	0	36	0	0
	Cutting ovaries	2	0	26	0	0
	Handling with contaminated hands	1	0	18	0	0
	Samuel's method	1	0	12	0	0
	Wedge insertion	5	5	45	39	87
	Hypodermic into nodes	2	0	28	0	0
	Hypodermic into internodes	2	2	28	14	50
	Hypodermic into sheathing leaves	2	2	26	13	50
	Hypodermic into internode, node above not cut	1	1	8	4	50
1932–33	Hypodermic into internode, node above cut	1	1	9	5	56
	Hypodermic into internode, internode above cut	1	1	9	7	78
	Hypodermic vertically into pith of cut stem	2	1	17	3	18
	Multiple needle through mosaic-diseased leaf	1	0	8	0	0
	Multiple needle through mosaic-diseased juice	1	0	12	0	0
1933–34	Hypodermic into internodes	4	4	53	38	72

According to the method of inoculation by wedge insertion, a wedge-shaped sliver is cut from the stem of a plant affected with mosaic and inserted into a simple longitudinal slit in the stem of a healthy plant. The wound is then covered with a wrapping of raffia or with a commercial wound dressing known as Tree Seal, to prevent excessive drying. This method was adapted from work with tulip mosaic. It proved useful in cross inoculations between varieties and in determining the significance of different classes of mosaiclike symptoms.

For studying properties of the virus, a method of injecting freshly extracted diseased juice into the stems of healthy plants by means of a fine hypodermic needle (Luer syringe no. 27) was developed by the junior writer.   This procedure yielded erratic results in earlier trials but performed consistently after the site of most effective inoculation was determined.

Other methods of inoculation have consistently failed.   These included (1) cutting the flower stalk of a healthy iris with a knife used for cutting a diseased flower stalk, (2) cutting healthy ovaries with a knife used for cutting ovaries of diseased plants, and (3) rubbing leaves of healthy irises with the hands contaminated from bruising leaves of diseased plants.   These trials indicate that iris mosaic is not likely to be spread from plant to plant in the field in the course of usual operations such as weeding, disbudding, or cutting flowers. No transmission resulted from multiple needle inoculations similar to Sein's,[5] whether the needles were forced through mosaic-diseased juice flooded on the healthy leaf or through a diseased leaf superimposed on the healthy leaf.   Samuel's [6] method of rubbing diseased juice into a healthy leaf by means of a roughened glass rod was also without effect.

Inoculations were made during the spring, while the irises were in bud or flower, and records were made from the progeny the following spring.   For example, in the growing season of 1929–30 inoculations were made in May 1929 and results were recorded in May 1930. Rows of sister-bulb controls derived from the same clump of the previous year as were the inoculated, bulb for bulb, were alternated with the inoculated rows in the same bed.   Controls on all the inoculations listed remained healthy the following season.   Either the three largest bulbs from the progeny of each inoculated and each control bulb were saved and grown for observation the following year, or the bulb clumps were left undug and observed in place.

In the seasons of 1929–30 and 1930–31 a number of inoculation trials listed as negative were attempts to transmit mosaic from plants showing atypical symptoms which the results indicated were not mosaic.   The percentage of plants infected in these seasons was calculated from the positive trials only.   During the seasons of 1931–32 and 1932–33 the inoculum was derived from known mosaic types, and the negative results therefore point to unfavorable factors in technique.   The percentage of infection for the trials of these years is based on the total number of plants inoculated.

Before the inoculations of the season of 1931–32 were begun, hypodermic injections of blue "script" ink were made into iris stems in the hope that the regions more readily penetrated by the ink would prove the more effective points for inoculation.   It was found that the pith of the internodes readily absorbed a large amount of ink, but that the spread from this region was not rapid.   Spread of the blue stain from the point of insertion was most extensive from node injections and from the midribs of leaves injected near the point of insertion on the stem.   Since leaf injections were cumbersome, it was decided to use node injection as a standard technique for the

[5] SEIN, F., JR. A NEW MECHANICAL METHOD FOR ARTIFICIALLY TRANSMITTING SUGAR-CANE MOSAIC. Jour. Dept. Agr. Puerto Rico 14: 49–68. 1930.
[6] SAMUEL, G. SOME EXPERIMENTS ON INOCULATING METHODS WITH PLANT VIRUSES, AND ON LOCAL LESIONS. Ann. Appl. Biol. 18: 494–507, illus. 1931.

season. The results recorded in 1932 indicated that the penetration of ink was not a good index of efficiency of inoculation, very low proportions of infection resulting from mosaic-diseased juice injected into nodes. Diseased juice injected into internodes in 1932 yielded much more favorable results, indicating that the internode is the most effective site for inoculation.

TRANSMISSION BY APHIDS

Since aphids were commonly found associated with apparent spread of mosaic in bulbous irises, transmission trials with these insects were undertaken in 1929. The original collections (except no. 76) were first colonized on healthy tulips under cloth cages in the greenhouse. The names [7] and sources of the aphid stocks used are given in table 3.

TABLE 3.—*Aphid stocks used in transmission tests*

Stock no.	Species	Source	
		Plant	Locality
11	*Illinoia solanifolii* (Ashm.)	Potato	California.
71	do	Iris	Grants Pass, Oreg.
77	do	Tulip	Corvallis, Oreg.
54	*Myzus circumflexus* (Buckt.)	Lily	Portland, Oreg.
10	*M. pelargonii* (Kalt.)	(¹)	Corvallis, Oreg.
75	do	Lily	Do.
50	*M. persicae* (Sulz.)	Tulip	Do.
53	do	Calla	Do.
51	*Rhopalosiphoninus tulipaella* (Theob.)	Tulip	Salem, Oreg.
76	*Anuraphis tulipae* (Boyer)	Iris [1]	Corvallis, Oreg.

[1] Greenhouse.

The cages used were of 40-mesh muslin on wood frames designed to cover two rows (20 bulbs) planted in Dutch beds. The cages were 2 feet high and had an opening at each end of the top surface which could be closed with a string tie when not in use. These cages were set in place in April before aphids were present on the irises. Sister-bulb controls, exactly comparable bulb for bulb to the caged sets, were grown uncaged, alternated with the sets caged for transmission trials. All progenies from these control plants remained healthy in 1930. Progenies from 29 healthy plants caged with mosaic-diseased plants in the absence of aphids during the 1929 season remained healthy in 1930. The uniform freedom from mosaic of these controls and of the lots of irises exposed to nonviruliferous aphids (table 5) shows that the cases of positive transfer of the virus (table 4) are attributable to the aphids named and that these aphids acquired the virus from the plants designated.

[7] Determined by P. W. Mason, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

TABLE 4.—*Transmission tests with aphids caged on mosaic-diseased irises and allowed to migrate to healthy irises under the same cage*

Aphid species and stock no.	Healthy iris variety exposed	Mosaic-diseased iris source of inoculum	Plants exposed in 1929 surviving in 1930	Mosaic-diseased plants, 1930	
				Number	Percent
Illinoia solanifolii, no. 11	Imperator	Imperator 104–1	13	2	15
Do	Hart Nibbrig	Hart Nibbrig	6	5	83
Do	Imperator	David Bles 163	31	3	10
Do	King of Yellows	King of Yellows	10	7	70
Do	King of Whites	King of Whites	3	3	100
Do	Therese Schwartze	Therese Schwartze	16	11	69
Do	Imperator	*Iris ricardi*	12	1	8
Do	do	*I. unguicularis alba*	2	1	50
Illinoia solanifolii, no. 71	do	Imperator 104–1	14	0	0
Do	King of Whites	King of Whites	4	1	25
Illinoia solanifolii, no. 77	Imperator	David Bles 163	11	5	45
Myzus persicae, no. 50	do	Imperator 104–1	16	11	69
Do	King of Whites	King of Whites	5	5	100
Myzus persicae, no. 53	Imperator	Imperator 104–1	11	1	9
Do	do	David Bles 163	18	8	44
Myzus pelargonii, no. 10	do	Imperator 104–1	14	0	0
Do	do	David Bles 163	12	0	0
Myzus pelargonii, no. 75	do	Imperator 104–1	17	0	0
M. circumflexus, no. 54	do	do	11	0	0
Do	do	David Bles 163	11	0	0
Rhopalosiphoninus tulipaella, no. 51.	do	Imperator 104–1	15	0	0
Do	do	David Bles 163	13	0	0

TABLE 5.—*Transmission tests in which aphids were transferred by camel's-hair brush from healthy and mosaic-diseased plants to healthy irises*

Aphid species and stock no.	Healthy iris variety exposed	Source of aphids	Plants exposed in 1929 surviving in 1930 [1]
Illinoia solanifolii, no. 11	Imperator	Tulip, healthy [2]	13
Do	do	Tulip, mosaic diseased	16
Do	do	Imperator 104, mosaic diseased	56
Do	do	David Bles 163, mosaic diseased	43
Do	Adrian Backer	Imperator 104, mosaic diseased	20
Do	D. Haring	do	15
Do	Frans Hals	do	12
Do	Golden Glory	do	15
Do	Leonardo da Vinci	do	18
Do	Queen Wilhelmina	do	18
Do	do	David Bles 163, mosaic diseased	17
Do	Rembrandt	Imperator 104, mosaic diseased	17
Do	S. Rombout	do	19
Do	Therese Schwartze	do	19
Illinoia solanifolii, no. 71	Imperator	Tulip, healthy [2]	17
Do	do	Tulip, mosaic diseased	13
Do	do	King of Whites, mosaic diseased	17
Illinoia solanifolii, no. 77	do	Tulip, healthy [2]	16
Myzus persicae, no. 50	do	do	12
Do	do	Imperator 104, mosaic diseased	16
Myzus persicae, no. 53	do	Tulip, healthy [2]	11
Do	do	Imperator 104, mosaic diseased	12
Myzus pelargonii, no. 10	do	Tulip and iris, healthy [2]	19
Do	do	Imperator 104, mosaic diseased	15
Do	D. Haring	do	18
Myzus pelargonii, no. 75	Imperator	Tulip, healthy [2]	10
Myzus circumflexus, no. 54	do	do	14
Do	do	Imperator 104, mosaic diseased	18
Rhopalosiphoninus tulipaella, no. 51	do	Tulip, healthy [2]	10
Do	do	Imperator 104, mosaic diseased	18
Anuraphis tulipae, no. 76	do	Forced iris, mosaic diseased (?)	14

[1] All exposed plants remained healthy in 1930.
[2] Nonviruliferous control.

Two types of transmission trials were made, with strikingly different results. Where aphids were colonized on diseased irises in the same cage with healthy irises and were allowed to migrate to the healthy plants, positive transfer of the virus resulted in several trials (table 4); but when aphids were transferred by means of a camel's-hair brush from diseased irises to healthy irises, there was no transmission (table 5). Similar failure of aphid vectors to transmit a virus when manually transferred had resulted from extensive tests of *Illinoia solanifolii* and *Myzus persicae* with tulip mosaic in 1928–29. It is a common experience to find manual transfer of aphid vectors less efficient than natural migration. This is usually attributed to some injury to the mouth parts of the insect in dislodging it from the leaf. Hoggan [8] has shown that the brush method is of low efficiency as a means of transferring *M. persicae* as a vector of cucumber mosaic, although the aphids were apparently not injured by the brush.

Transfers were made to healthy tulips under cloth cages in the field to serve as nonviruliferous controls on tulip mosaic studies and as general stocks for both tulip and iris tests. Subcultures from these colonies were placed on healthy irises, as a test of freedom from the iris mosaic virus, and other lots were colonized on irises affected with mosaic. Such colonies on diseased irises were allowed to feed and multiply for at least 2 weeks before further transfer to healthy irises for testing.

It is evident from table 4 that *Illinoia solanifolii* and *Myzus persicae* can act as vectors of iris mosaic. Three stocks of *I. solanifolii* transmitted mosaic to 32 percent, 6 percent, and 45 percent of the plants exposed; two stocks of *M. persicae* transmitted mosaic to 76 percent and 31 percent of the plants exposed. *M. pelargonii*, *M. circumflexus*, and *Rhopalosiphoninus tulipaella* failed to transmit iris mosaic in any test, although the diseased plants used as sources of inoculum were comparable to those used in the positive trials with the two vectors mentioned above, and in some trials these species thrived on the diseased plant and migrated to healthy plants to an extent sufficient to make the tests seem adequate. In one trial *Anuraphis tulipae* was transferred from forced Dutch irises containing some mosaic to healthy irises but did not multiply on the latter; similar manual transfers of the two species capable of acting as vectors (table 5) indicate that this test was entirely inadequate.

The great variation in efficiency of *Illinoia solanifolii* and *Myzus persicae* in the several trials shown in table 4 seems explainable largely by the amount of natural migration from the diseased to the healthy plants. In the long, narrow cages used it was hardly likely that aphids would move naturally to all the plants caged unless the infestation was very heavy. Where natural migration was slow, aphids were moved to more distant plants under the cage in some instances, but the results of manual transfer in other tests (table 5) indicate that such moving was not likely to be effective.

Twenty-eight collections of aphids from the leaves and flower buds of bulbous irises included five species [9] distributed as follows: *Illinoia solanifolii* from 15 iris plantings (Washington, 3; Oregon, 10; California, 2); *Myzus persicae* from 5 (Washington, 1; California, 4);

[8] HOGGAN, I. A. SOME FACTORS INVOLVED IN APHID TRANSMISSION OF THE CUCUMBER-MOSAIC VIRUS TO TOBACCO. Jour. Agr. Research 47: 689–704, illus. 1933.
[9] Determined by P. W. Mason.

Macrosyphum sp. from 4 (Washington, 2; Oregon, 1; California 1); *Anuraphis tulipae* from 3 (California); *Aphis* sp. from 1 (California). All collections from Washington bulb plantings were made in mid-May of 1928. California collections were made in late February and early March 1929. All collections in Oregon were made during May, except that *I. solanifolii* was once taken from iris at Grants Pass, Oreg., on February 1. The two species of aphids that have been shown to be vectors of iris mosaic appear to be sufficiently common and widely distributed on bulbous irises, especially when these are in bud, to account for the natural spread of the disease. A paper by Whitaker [10] confirms and extends some of the writers' findings with respect to aphids as vectors of iris mosaic.

<center>HOST RANGE</center>

The bulbous irises here discussed include varieties derived from the species *Iris filifolia* Boiss., *I. tingitana* Boiss. and Reut., *I. xiphium* L. (Spanish iris), and *I. xiphium praecox* Hort. (Dutch iris), all of which are included in the section Xiphium. Cross inoculations between varieties ascribed to these species have been readily successful. In the trials that have been made the wedge-insertion and hypodermic methods and the two aphid vectors transmitted mosaic from one species to another as freely as from plant to plant within a variety. The varieties named below are Dutch irises unless otherwise designated.

Imperator was successfully inoculated with mosaic from Celestial, David Bles (Tingitana type), Hart Nibbrig, Huchtenburg, Imperator, King of Whites (Spanish), J. W. de Wilde, Leonardo da Vinci, Therese Schwartze, Wedgewood (Tingitana), and Yellow Queen. Mosaic from Imperator was successfully transferred to Adrian Backer, Celestial, Golden Glory, Hart Nibbrig, Huchtenburg, Hobbema, Leonardo da Vinci, S. Rombout, Rembrandt, Queen Wilhelmina (Spanish), and Yellow Queen. Inoculations also were successful from David Bles (Tingitana) to Hobbema and Leonardo da Vinci, and from King of Whites (Spanish) and Therese Schwartze to Golden Glory. The failure of one attempt to transmit mosaic from The First (Tingitana) and of several attempts to transmit from David Bles (Tingitana) was probably due to the difficulty of distinguishing mosaic symptoms from other confusing patterns in varieties derived from this broadleaved species rather than to any difficulty in transmitting the mosaic virus. All commercial varieties of bulbous irises tested have been found susceptible, but differences in degree of injury are apparent.

In the season of 1929–30, mosaic was successfully transmitted to Imperator from *Iris ricardi* (section Pogoniris) and from *I. unguicularis* Poir. var. *alba* Hort. (section Apogon). *Illinoia solanifolii* was colonized on potted plants of these species, and the pots were set under cages with healthy Imperator. The resulting mosaic in Imperator has been subinoculated to bulbous irises in later seasons and appears to be the same disease that was found in the bulbous types. The original collections of mosaic-diseased *I. ricardi* and *I. unguicularis alba* were from Redlands, Calif. Attempts to transmit a virus from mosaiclike mottling in German or bearded iris hybrids have not

10 WHITAKER, W. C. THE POSSIBILITY OF INTER-TRANSMISSION OF TULIP MOSAIC BY INSECT VECTORS, ESPECIALLY APHIDS. Thesis, Oreg. State Col., 26 pp. 1931. [Unpublished.]

been uniformly successful, but mosaic was transmitted to Therese Schwartze from strongly mottled William Mohr in 1934–35.

All attempts to transmit iris mosaic by inoculation into plants outside the genus and all attempts to infect irises with viruses from other genera have failed. These cross inoculations included iris mosaic to Turkish tobacco by leaf rubbing, to tomato and petunia by the carborundum method, and to tulip by wedge insertion and by *Illinoia solanifolii* (manual transfer); tulip mosaic to iris by *I. solanifolii* (natural migration); and lily mosaic to iris by wedge insertion and by hypodermic injection.

DISCUSSION

As pointed out in the description of symptoms, there is great variability in both type and intensity of the disease in inoculated irises. This variability is expressed in the degree of dwarfing and the intensity of flower break and of leaf mottling. In some instances the symptoms of a parent plant have been consistently reproduced in its vegetative progeny for two or more seasons. In other individual plant progenies the severity of symptoms, particularly in the flowers, has changed from year to year.

On subinoculation from plants showing strongly mottled leaves and strongly broken flowers with typical teardrop markings the same type is reproduced in the inoculated plants in the majority of cases, but individuals with mottled leaves and unbroken flowers also appear. In several inoculations both types have appeared within the same inoculated group. When inoculations have been made from individuals with typical mosaic-affected leaves but apparently normal flowers the plants inoculated have developed typical broken flowers more frequently than the symptom type used as inoculum. The intensity of the leaf symptoms in inoculated plants is commonly similar to that of the source plant, but in some instances a mild mottling has resulted from a strongly mottled source.

There is little to indicate that the methods of inoculation used will prove selective if a complex of viruses is involved, inasmuch as severe mosaic mottling of the leaves, intense flower breaks, and extreme dwarfing have all appeared in progenies inoculated by the hypodermic-needle method, by wedge insertion, and by the two vectors *Myzus persicae* and *Illinoia solanifolii*. More detailed experimental analysis, involving the use of seedlings, is needed before the nature of the several types of symptoms can be explained.

The movement of the mosaic virus within the group of bulbs developed by a single plant during the growing season is rapid. If a plant is inoculated during the bud or flowering stage in May and the bulbs are dug and separated in July, the progeny are as a rule all affected with mosaic or are all healthy in the following season. In individual plant progenies from 29 of the positive inoculations made in 1929 and recorded in 1930, all members of the group were affected with mosaic in 137 plant units, 6 units segregated into 1 healthy and 1 diseased, and 1 unit into 1 healthy and 2 diseased. Three segregating units were from plants inoculated by aphids, and four from plants inoculated by wedge insertion. The three largest bulbs were saved from each plant inoculated in 1930. The resulting units grown in 1931 were uniformly diseased or uniformly healthy. Although these

records are based on the performance of the larger bulbs in a plant unit, it appears that the progenies of plants infected as late as the last week in May are likely to be uniformly diseased when grown the following season.

SUMMARY

A mosaic disease of iris was found widely distributed in Washington, Oregon, and California as early as 1928 on bulbous irises originally imported from the Netherlands.

The symptoms of the disease include mosaic mottling of the leaves and the bud sheaths, "breaking" of the flowers, and general dwarfing.

Epidermal cells of mosaic-affected leaves are smaller than normal leaf cells, and intracellular inclusions of vacuolate or reticulate types sometimes occur. The number of plastids is reduced in epidermal cells in flecked areas of flowers affected with mosaic.

The virus is transmissible by insertion of a wedge of diseased stem tissue or by injection of diseased juice by means of a fine hypodermic needle. Other mechanical methods of transfer have been ineffective so far as tested.

Illinoia solanifolii and *Myzus persicae* are able to act as vectors of iris mosaic. *M. pelargonii*, *M. circumflexus*, and *Rhopalosiphoninus tulipaella* failed to transmit the virus in parallel tests.

Cross inoculations between different colored varieties in the bulbous irises and between varieties of Dutch and Spanish irises are readily successful.

Mosaic has been transmitted to bulbous irises from naturally infected *Iris ricardi*, from *I. unguicularis alba*, and from the bearded iris William Mohr.

Attempts to transmit iris mosaic to tobacco, tomato, petunia, and tulip and attempts to infect iris with mosaic from lily and tulip have been unsuccessful.

Attention is called to several symptom types included in iris mosaic that have not yet been satisfactorily explained.

HISTOLOGICAL AND CYTOLOGICAL CHANGES IN SUGAR-BEET SEEDLINGS AFFECTED WITH CURLY TOP[1]

By ERNST ARTSCHWAGER, *pathologist*, and RUTH C. STARRETT, *assistant cytologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

Virus diseases, because of their weakening effect on the organism, are responsible for many stunted plants with greatly impaired reproductive capacity. The developmental disturbances are local or systemic, most easily noted in young, growing tissue, and practically absent from mature organs. During the early stages there are cytoplasmic and nuclear changes and, in the aerial parts, disturbances in the chlorophyll apparatus.

Changed nuclear forms have been observed in connection with many virus diseases, notably mosaic, but their significance has been variously evaluated. Thus, Goldstein (*12*)[3] finds the nuclei of mosaic plants hardly changed, whereas Schander and his coworkers (*16*) consider mosaic a nuclear disease entirely. A changed cytoplasmic structure, caused by extreme vacuolization and accompanying proteolysis, has also been considered an important symptom in young cells (*6*). Last but not least, the X-bodies, first discovered in mosaic plants by Iwanowski (*13*), raised high hopes and started a deluge of investigations into their nature. Once thought by some to be the cause of virus diseases, they are now mostly relegated to the position of some obscure metabolic product of the virus-infected cell.

Curly top of sugar beets (*Beta vulgaris* L.) belongs to a group of virus diseases of major economic importance, but, as in the case of all virus diseases, little is known about its nature. To be sure, much information has recently been gained from the work of Esau (*8, 9, 10, 11*) and Bennett (*4*). Thanks to their studies, we have a better understanding of the minute structure of diseased and normal phloem as well as the location and movement of the virus. Little is known as yet about the early cytological responses following infection, and it was with this in mind that the studies recorded in this paper were undertaken.

Investigations on the seedstalk of curly top beets were carried on for several seasons, but subsequent studies on diseased roots have yielded more valuable knowledge concerning cellular responses to virus invasion. The seedling root possesses a large amount of phloem, while the simultaneous activity of the supernumerary cambium provides a wealth of relatively young cells for the virus to act upon. Practically all of these immature cambium derivatives mature

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into phloem tissue and are open to virus invasion; interzonal parenchyma cells, being far less subject to disturbance by abnormal stimuli, are not differentiated until later. There are also few complications arising from the effect of secondary factors, such as disturbed chlorophyll apparatus, phloem exudate channels, and insect injuries.

Shortly after these studies were completed there appeared a paper by Esau (*11*), part of which, dealing with degeneration of the primary root, is an elaboration of her own preliminary investigations (*8*) and those of Rawlins (*15*). While there is some duplication in these studies and those recorded in the present paper, emphasis, on the whole, is put on widely different topics which make the studies complementary to each other rather than competitive.

MATERIALS AND METHODS

Most of the material for study was obtained through the cooperation of the field station of the United States Department of Agriculture at Riverside, Calif., and consisted of beet seedlings of strain 2769, very susceptible to curly top, and of the resistant strain 286. The plants were grown in pots in the greenhouse; to obtain diseased plants, virus-carrying leafhoppers were caged upon individual leaves and were removed several days later. The material was killed in several fixing fluids, and the usual methods of dehydrating and embedding in paraffin were employed.

The stain most successfully employed was Flemming's triple stain, used according to the following modified schedule: [4]

(1) Slides, with paraffin removed, in 50-percent alcohol.
(2) Stain in safranine 1 hour. (The stain is prepared by mixing equal parts of a 1-percent solution of water-soluble and alcohol-soluble safranines.)
(3) Transfer to water.
(4) Dip into acidulated 70-percent alcohol.
(5) Wash for a brief time in 70-percent alcohol.
(6) Dip into 85-percent alcohol.
(7) Dip into 95-percent alcohol.
(8) Transfer to absolute alcohol.
(9) Transfer to a mixture consisting of one-third absolute alcohol and two-thirds xylol; change twice.
(10) Stain in crystal violet 5 seconds or longer. (Prepare stain by mixing 2 cc of saturated crystal violet in clove oil with 1 cc of absolute alcohol and 50 cc of xylol.)
(11) Transfer to xylol; change four times.
(12) Stain in orange G on slide; follow staining reaction under microscope and interrupt staining at proper stage. (Stain is prepared by making a saturated solution of the powder in clove oil, filtering, and adding a few drops of xylol. Only the best orange G, preferably Grübler's, should be used to obtain uniformly good results.)
(13) Transfer to xylol; change several times.
(14) Mount in balsam.

The Feulgen chromatin reaction as given by Lee (*14*) was used in a somewhat modified form according to the following schedule:

(1) Slides, with paraffin removed, in water.
(2) Treat with normal hydrochloric acid at 60° C. for 12 minutes.
(3) Dip in water.
(4) Stain in colorless B fuchsin for 3 to 4 hours. (The stain is prepared as follows: Dissolve 1 g of basic fuchsin in 200 cc of hot water; cool to 50° C. and filter; cool down to 25° and add 1 g of potassium metabisulphite. Keep solution in the dark in a tightly stoppered bottle for at least 24 hours before using.)
(5) Transfer to a freshly prepared solution of: Water, 200 cc; normal hydrochloric acid, 10 cc; potassium metabisulphite, 1 g. Keep slides in this solution for 6 minutes.

[4] Recommended by D. M. Weller, of the University of Hawaii.

(6) Wash in running water 10 minutes.
(7) Transfer successively to 60-, 80-, 95-, and 100-percent alcohol.
(8) Transfer to xylol.
(9) Mount in balsam.

In the Feulgen reaction, the hot hydrochloric acid decomposes the thymonucleic acid contained in the chromatin. The aldehyde that is liberated forms with the leucobase of basic fuchsin an addition compound which is colored a vivid cherry red.

The material used for the Feulgen reaction was killed in a concentrated aqueous solution of corrosive sublimate to which 2 percent of acetic acid was added.

All photographs were taken on Wratten M plates with B-58 and E-22 filters used singly or in combination. The drawings[5] are mostly based on photomicrographs.

<h2 style="text-align:center">STRUCTURE OF NORMAL AND DISEASED TAPROOT</h2>

Since a detailed description of structure and development of beet roots is given elsewhere (*1*), it will suffice here to recall only the salient features. Microscopically the young beet seedling shows three regions—root, hypocotyl, and cotyledons. The junction between hypocotyl and root is indicated by an abrupt tapering of the axis and the appearance of lateral rootlets. The center of the root is occupied by a thin strand of vascular tissue, which is enclosed by a cortex and bound peripherally by a single-layered epidermis. The central cylinder of root and lower hypocotyl shows a diarch protoxylem plate with alternating phloem groups, a single-layered pericycle, and a band of parenchyma between xylem and phloem. The innermost layer of these parenchyma cells gives rise to the primary cambium, which as such develops xylem and phloem in the normal manner. Before primary growth is completed, there arises in the tissue between endodermis and primary phloem the first secondary cambium. It is derived either directly from cells of the pericycle and primary phloem parenchyma or from their descendants. The second supernumerary cambium is formed from the outer daughter cells of the first cambium initial, and additional cambiums are derived in a similar manner. In a longitudinal section of a beet root, the cells of the secondary cambiums and their derivatives have a characteristically tiered arrangement. They are very uniform, of similar size, and, when mature, make up the sieve tubes, companion cells, and parenchyma of the secondary phloem.

The phloem parenchyma cells are larger than the sieve tubes and have straight or slightly inclined end walls. They retain their nuclei and cytoplasm and may contain leucoplasts and even starch. The companion cells also possess nuclei but are usually shorter and always narrower than the phloem parenchyma cells. The sieve tubes lack nuclei and appear empty. They have well-developed terminal sieve plates, sieve-tube plastids, and, when young, variously shaped slime bodies such as those described by Esau (*10*) for the sieve tubes of the leaf.

As compared with the normal structure just described, the picture of a radial section through a root affected by curly top differs at first glance by showing a disturbance of the typically tiered arrangement

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[5] All the drawings were prepared by Mrs. Eugenia Artschwager.

of the cells caused by the interpolation of hypertrophied and diseased cells among the normal ones. Some of these cells have enlarged uniformly, while in others the longitudinal and transverse diameters increased unequally, giving rise to greatly elongated or irregular cubical cells. Most of these hypertrophied elements are phloem parenchyma cells. They possess nuclei, however greatly changed at times, and almost all of them have peripheral cytoplasm. For the most part they are empty but may contain as transitory accompaniments abnormal amounts of leucoplasts, crystal sand, and inclusion bodies.

Scattered among the elements of the diseased secondary phloem are certain cells or cell groups which a closer study reveals to be sieve-tubelike elements. They are axially elongated, like the surrounding cells, and their end walls are for the most part straight. Occasionally the cells are pointed or quite irregular. These aberrant cell forms must be produced as a result of direct or indirect stimulation from the curly top virus, since they are wanting in normal beet tissue. Characteristic of these sieve-tubelike cells is the abundance of large sieve-tube plastids and slime bodies (pl. 1, *A–D*) and the lack of nuclei and terminal sieve plates. Withal, the tissue resembles in both origin and structure the hyperplastic cell groups recently described and illustrated by Esau (*9*) for the diseased phloem of young beet leaves. The homologous nature of these pseudosieve tubes in roots and aerial organs is confirmed by a study of the effect of curly top virus on the internal structure of young leaves and of the seedstalk of flowering beets. Plate 2 gives the sequence of development of these pseudosieve tubes in the midribs of seedling leaves. The walls of the potential hyperplastic cells are not only thickened and chemically changed, as indicated by the staining reaction, but this thickening had taken place before there was any recognizable pathological disturbance in either nucleus or cytoplasm. Details for the formation of the hyperplastic tissue are given in Esau's paper, and only such observations are here recorded as seem necessary for the general understanding of the early cytological phenomena associated with the disease.

STRUCTURE OF NORMAL AND DISEASED LATERAL ROOTLETS

The lateral roots of the beet are fibrous and covered for a considerable distance with root hairs. They arise endogenously in the pericycle of the young taproot, mostly opposite the two protoxylem points, and thus appear externally in two opposite longitudinal rows. Structurally the lateral roots differ from the taproot in the very limited amount of secondary growth, the absence of supernumerary cambiums, and the occurrence among them of triarch and tetrarch forms besides the traditional diarch ones.

Although the number of laterals is normally large, there is a further increase in beets affected with curly top. The rootlets appear here very crowded, so much so that often two laterals arise from the same initial. As active organs of absorption, however, they have only a limited existence. The increase in the number of lateral rootlets in diseased plants may be considered a physiological response whereby new organs of absorption are formed, replacing those prematurely dead.

A, Young irregular hyperplastic cells from taproot of seedling beet, showing numerous slime bodies and sieve-tube plastids. Cells at left still possess nuclei. × 1,300. *B*, Narrow hyperplastic sieve-tubelike cells with numerous small slime bodies. × 710. *C*, Slime bodies and sieve-tube plastids. At right, large cleavage nucleus. × 900. *D*, Hyperplastic sieve-tubelike cells with numerous large sieve-tube plastids but without terminal sieve plates. × 1,300.
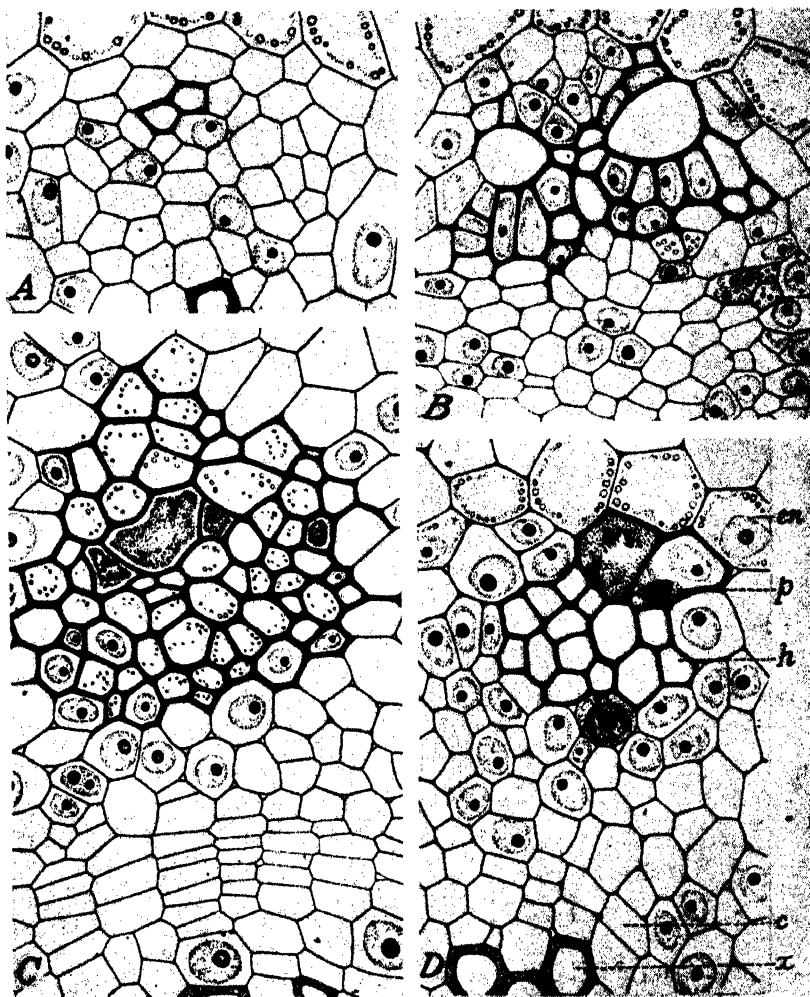
A, Cross section of midrib of embryonic leaf with two sieve tubes and one protoxylem element. The sieve tubes are separated from the endodermis by two rows of pericyclic cells. *B*, Cross section of midrib of leaf somewhat older than the one illustrated in *A*. One pericyclic cell has greatly enlarged; the potential hyperplastic tissue is indicated by the great thickness of the walls of the cells. *C*, Cross section of midrib of a still older leaf. The hyperplastic tissue is quite massive; most of the cells contain numerous sieve-tube plastids. *D*, Cross section of a lateral bundle from leaf illustrated in *C*. Two of the pericyclic cells are enlarged and their content has degenerated; the hyperplastic tissue is extensive. *en*, Endodermis; *p*, pericycle; *h*, hyperplastic tissue; *c*, cambium; *x*, xylem. The position of the endodermis in *A*, *B*, and *D* is indicated by its content. All × 748.
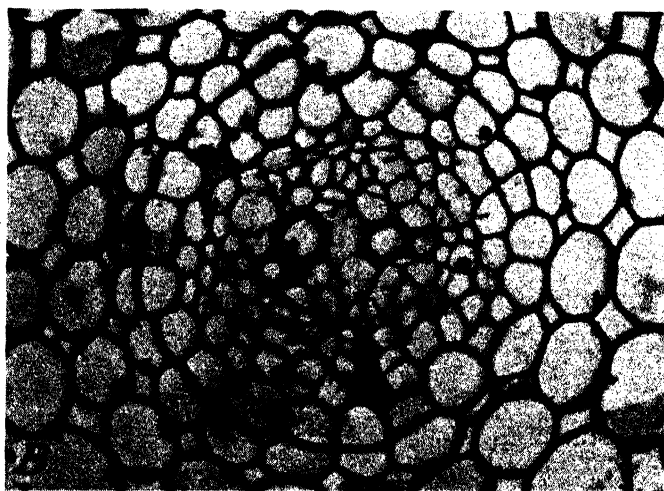
A, Longitudinal section of taproot and lateral rootlet. The lateral rootlet leads vertically downward through the cortex before emerging. × 95. B, Transverse section of a lateral rootlet with some of the cells diseased. × 810.
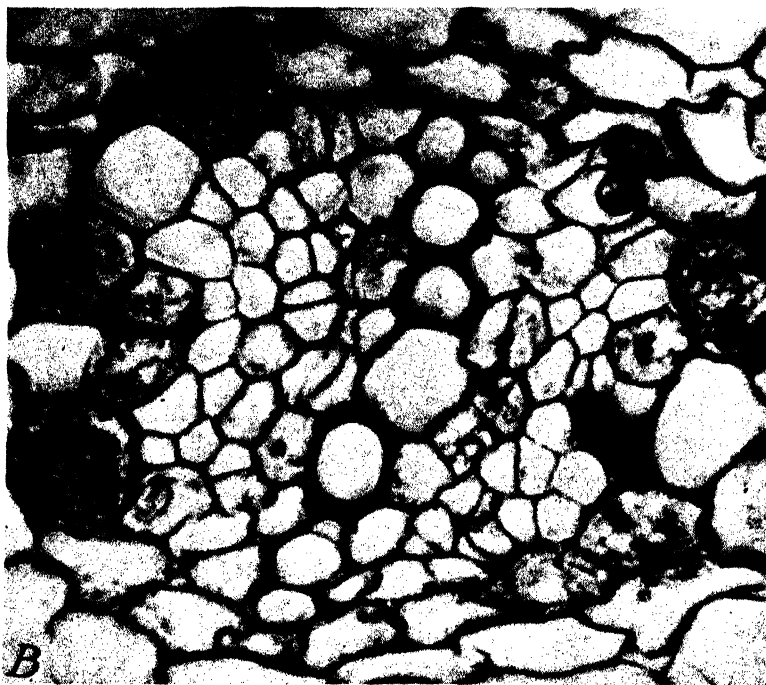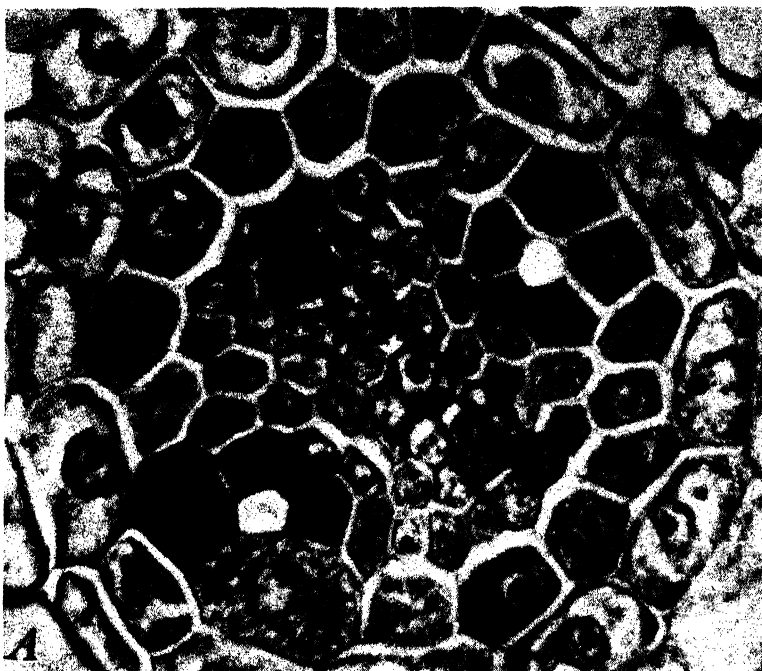
A, Transverse section through tip of lateral rootlet. Notice the two sieve tubes devoid of content; also enlargement in the adjacent pericyclic cells. × 1,400. B, Transverse section of diseased lateral rootlet. The pericyclic cells are greatly enlarged and the nuclei hypertrophied and in the process of degeneration. × 1,400.

The peculiar behavior on the part of some of the laterals in breaking through the cortex is of interest in this connection. Instead of pursuing a normal horizontal or somewhat oblique course, they continue vertically downward in the tissue of the cortex before finally breaking through the epidermis. The downward course of such a lateral is illustrated in plate 3, *A;* while *B* of the same figure gives the anatomical structure of such a rootlet in cross section. The cortex of a young rootlet is composed of only a few rows of large cells, which make a relatively broad ring around the miniature stele. The pericycle is one cell wide and abuts directly on phloem and protoxylem. The phloem appears in two oblong groups in juxtaposition. It is surrounded, except for the region of the pericycle, by more or less undifferentiated procambium. The latter is to all purposes identical with phloem parenchyma and companion cells, and only the two protophloem sieve tubes (pl. 4, *A*) are readily recognized as such.

The lateral rootlets become infected through the taproot. Since their structure is relatively simple, it is easier to trace the effect of the curly top virus here than in the complicated structure of the main root. The most striking changes, as seen in plate 4, *B*, are exhibited by the cells of the pericycle. These cells are greatly enlarged and undergo subsequent degenerative changes as the disease increases in severity. The primary sieve tubes become occluded with a red-staining gummy substance or they appear completely collapsed with only the walls showing. Degeneration of the primary sieve tubes, however, is a normal phenomenon, and the process in diseased roots may only be hastened and otherwise slightly changed. Depending on the severity of the disease, other tissues, including cortex and endodermis, become involved; and in the extreme form of the disease the rootlet becomes reduced to a necrotic mass of cells in which the original structure is hardly discernible.

DEVELOPMENT OF CALLUS AND PSEUDOCALLUS

Diseased taproots of young beet seedlings often show peculiar beadlike protuberances on the walls of the sieve tubes and sieve-tube-like elements described above. These swellings are found on both terminal and lateral walls. They vary greatly in number and size. Occasionally only terminal swellings are found, but more often lateral ones are also present. The terminal swellings are almost always massive, while those along the lateral walls are small or medium, and, since they normally appear on opposite sides of the same wall, they impart to the latter a peculiar beaded appearance (pls. 5, *A;* 6, *F*). Where sieve tubes and pseudosieve tubes border on pericyclic or phloem parenchyma cells, swellings are found only along the walls of the sieve tubes and pseudosieve tubes (pl. 6, *C*). Large lateral swellings are commonly found near the end of the cell, but may occupy any place along the lateral wall.

The swellings at first glance suggested callus formations, but subsequent studies showed them to be different in origin and composition. Normal callus (pl. 6, *A, B*) appears rather early in the primary sieve tubes of the root. Like any normal callus, it first shows as a uniform deposit over one side of the sieve plate, plugging the pores of the latter. The callus deposit, quite thin at first, becomes massive (*B*), effectively sealing the terminal wall of the now functionless sieve tube.

Compared to the normal callus, the wartlike swellings just described differ in being equally massive on either side of the sieve plate, in developing in places where normal callus is not formed, and, finally, in not giving the characteristic microchemical callus reaction.   For the sake of convenience the calluslike wall swellings will be referred to as "pseudocallus."

The terminal pseudocallus appears as an abnormal wall swelling which is faintly divided by the differently staining middle lamella (pl. 6, *H*) into two more or less equal parts.   The outer part of the swelling is always much darker than the inner substance, but old swellings may be uniformly dark throughout (*G*).   The developing terminal callus often stains brightly with orange G, and the matrix of the thickened part is foamy or spongy (*E*) instead of having the consistency of a homogeneous gel.   Another frequently observed form of terminal callus is shown in *D*.   It looks like a large blister compressed into the lumen of the rather narrow element; the swelling is divided by a thin, indistinct middle lamella.   The body of the swelling appears as a homogeneous substance bounded peripherally by a dark crust.   This peculiar callus formation may be observed in all types of material, but it is relatively conspicuous in resistant varieties, since in these abnormalities of other types are rare.

The lateral pseudocallus is structurally quite uniform, the outer shell taking a deeper stain than the central zone.   It may be found in any stage of development, with small and massive forms occurring side by side on the same cell wall.

Whether developed terminally or along the side walls, the pseudocallus is initiated as a small discoloration of the outer lamella of the primary wall as seen in plate 5, *A–C*.   The discolorations in the terminal wall soon become confluent, but the lateral swellings remain localized or may unite incompletely if development is extensive.   The discolored areas gradually thicken, assuming varied forms, as shown in plates 5 and 6.   Their ontogeny is controlled by the metabolism of the host cell, whose life in turn is affected by many factors such as age, relative position within the tissue, degree of stimulation (by the virus or its products), and food supply.

The formation of pseudocallus is one of the earliest reactions on the part of the host to curly top infection.   It is observed before the advent of abnormal cell division and becomes conspicuous after the development of hyperplastic sieve-tubelike tissue is in progress.   It is found frequently in resistant plants where internal symptoms are often altogether wanting.   The swellings are naturally more pronounced in the taproot of seedlings than in the lateral rootlets, since the latter develop relatively little secondary tissue and rarely ever hyperplastic cells.

NORMAL AND ABNORMAL LONGITUDINAL DIVISION

The axially elongated cells of the sugar-beet rootlet, notably those of the pericycle and cambium, divide longitudinally after a pattern described by Bailey (*2*) for the cambium cells of the white pine.   In beets affected with the curly top disease, where cells of abnormal dimensions are not uncommon, this peculiar type of division is found not only in axially elongated cells but also in relatively short elements; in fact, in cells which may be all but isodiametric and where the usual type of cell-wall formation should be the rule.

A, Transverse section of phloem of first supernumerary ring of diseased seedling showing pseudocallus in all stages of development. × 940. *B*, Advanced stages of pseudocallus formation. × 1,360. *C*, initial stages of terminal and lateral pseudocallus formation. × 1,360.

A, Normal sieve-tube callus in young sieve tube. B, Older callus in mature sieve tube. C, Terminal and lateral pseudocallus. Notice its absence from the walls of the phloem parenchyma cells. D, Massive terminal pseudocallus. E, Spongy terminal pseudocallus. F, Beaded appearance of lateral pseudocallus. G, Old lateral and terminal pseudocallus, very homogeneous in nature and staining almost black. H, Greatly enlarged old terminal callus. All × 1,450.

During telophase of such longitudinal division, the spindle substance widens in the equatorial region to form a barrel-shaped phragmoplast, which then extends laterally until it comes in contact with the end walls of the cell. During this process the spindle substance soon disappears near the nucleus but continues at the margin of the growing cell plate by the addition of accessory fibers that make up the two secondary phragmoplasts. The cell plate, according to present-day concept (*17, p. 174*), appears as a liquid film at the equator at the close of mitosis; the deposition of salts and other matter in the film transforms it into the middle lamella. The secondary phragmoplasts are located halfway between the tangential walls of the cell and are usually equidistant from the daughter nuclei (pl. 7, *A, C*). This symmetrical arrangement is not always observed with this type of division, for the distance of each of the daughter nuclei frequently shows variation in relation to the approximate center of the protoplast. The phragmoplasts, as described by Bailey for the white pine, have a wedge-shaped outline, bluntly convex in front and tapering to a point along the rear of the cell plate. In sugar beets they have the form of a blunt cone (*A*), with the flat side in the direction of the nucleus. From this flat side the fibers taper rather gradually along the cell plate and finally disappear.

There is little variation in the size of the phragmoplast, except that cells which have a large primary spindle tend to have larger phragmoplasts, and these are commonly, though not always, found in large cells. The fibers of the phragmoplast closely resemble those of the normal spindle. Of course, they are much shorter than the normal fibers and extend only partly across the cell lumen. The terminal portions of the cell plate, with the two phragmoplasts, travel to their respective ends of the cell, leaving a fully formed cell plate behind them. The latter is extended until the cell is completely divided into halves, each of which contains one of the daughter nuclei.

Binucleated cells are occasionally found in healthy beet roots, but they are of common occurrence in plants affected with the curly top disease. According to Beer and Arber (*3*), binucleated cells are frequently found in growing tissue of higher plants. These investigators also found certain peculiar features connected with the mitosis and behavior of the associated protoplasm, a behavior which Bailey (*2*) later encountered and illustrated in his study of division in the cambium cells of the white pine. It appears that the daughter nuclei in telophase were found to be enclosed in a hollow sphere of dense protoplasm, the appearance suggesting a cell within a cell. Bailey strongly suspected the phenomenon referred to by Beer and Arber to be a phase of cytokinesis not unlike that illustrated by him.

Since the root tissue of beets offers an unusual opportunity for the study of this peculiar and probably little known phenomenon, it might not be out of place to give a brief account of it in this paper.

The initiation of cell division leading to the binucleated condition follows the normal course. There is nothing unusual in the appearance of the metaphase spindle or in the succeeding stages except that in late anaphase the cytoplasm concentrates rimlike about the karyokinetic figure (pl. 7, *E*). With the disappearance of the spindle fibers and the reconstruction of the daughter nuclei, this rim of kinoplasm becomes quite distinct, forming, to use the words of Bailey (*2*), a halo around the daughter nuclei (*B, II*). In cells of

large diameter, this rim of kinoplasm widens, and when it intersects
the radial walls of the cell it becomes more or less angular.   There is
no wall formation in progress between the daughter nuclei.   The
kinoplasm now disappears from the side walls of the rectangular rim,
leaving two granular masses which extend from one radial wall to the
other (*D*, *G*).   The granular masses of fibrillae, comparable to the
phragmoplasts of the normal longitudinal-division figures, now move
in opposite directions (*D*) until they eventually reach the ends of
the protoplast.   The office of the phragmoplasts in the formation of
the new wall in cells dividing longitudinally is clear, even though we
may not comprehend the mechanism by which this is accomplished;
it would be futile, however, to attempt a reasonable explanation for
the rather complicated method of division in cells where cell-wall
formation between the daughter nuclei is never realized even in the
initial stages.

The course of reconstruction of the daughter nuclei resulting from
this type of division is usually belated.   The chromatin often remains
in the form of chromosomes (pl. 7, *F*) long after the phragmoplasts or
their equivalents have receded from the daughter nuclei and are
well on their way, moving in opposite directions toward the ends of
the cell.   Nuclei formed in this peculiar manner occasionally possess
a larger chromosome complex; even tetraploidy has been observed
in them.

REACTION OF THE NUCLEUS TO CURLY TOP INFECTION

CHANGES IN SIZE AND FORM

Giant nuclei are of common occurrence in beets affected by curly
top.   The cells in which they are found are also enlarged, though
nuclear hypertrophy may be observed in cells of normal size, in
which case the nucleus fills the greater part of the cell lumen.

The enlarged nuclei are mostly round.   Other nuclear forms are
largely bound up with the shape of the cells; thus, in greatly elongated
cells, the nuclei are also exceptionally long.   On the other hand,
spherical nuclei are also observed in elongated cells (pl. 8, *A*) and are
found almost without exception in all irregular cell forms.

Cells with enlarged nuclei belong morphologically to the pericycle
or the phloem parenchyma, depending on the age and type of root.
Thus, in young laterals the cells of the pericycle are hypertrophied,
whereas in taproots, where secondary growth has already become
pronounced, the nuclei of the phloem parenchyma are those mostly
affected.

Increase in nuclear size may occasionally be brought about by
nuclear fusion, the process being often facilitated through the multi-
nucleated condition of the affected cells.   Such compound nuclei,
however, are readily recognized, even though the separate nuclear
membranes have disappeared.   (See pl. 11, *J*.)

Amoeboid nuclei are not uncommon.   They are often observed in
cells of endodermis and cortex but rarely ever in the cells of the
vascular tissue.   Edson (7) described and illustrated such irregular
nuclear forms in beet seedlings affected with *Phoma betae*, but did
not indicate in what tissue they occurred.

In the process of hypertrophy, nuclei may become irregular,
notched, cleft, or deeply segmented.   An extreme type of this group
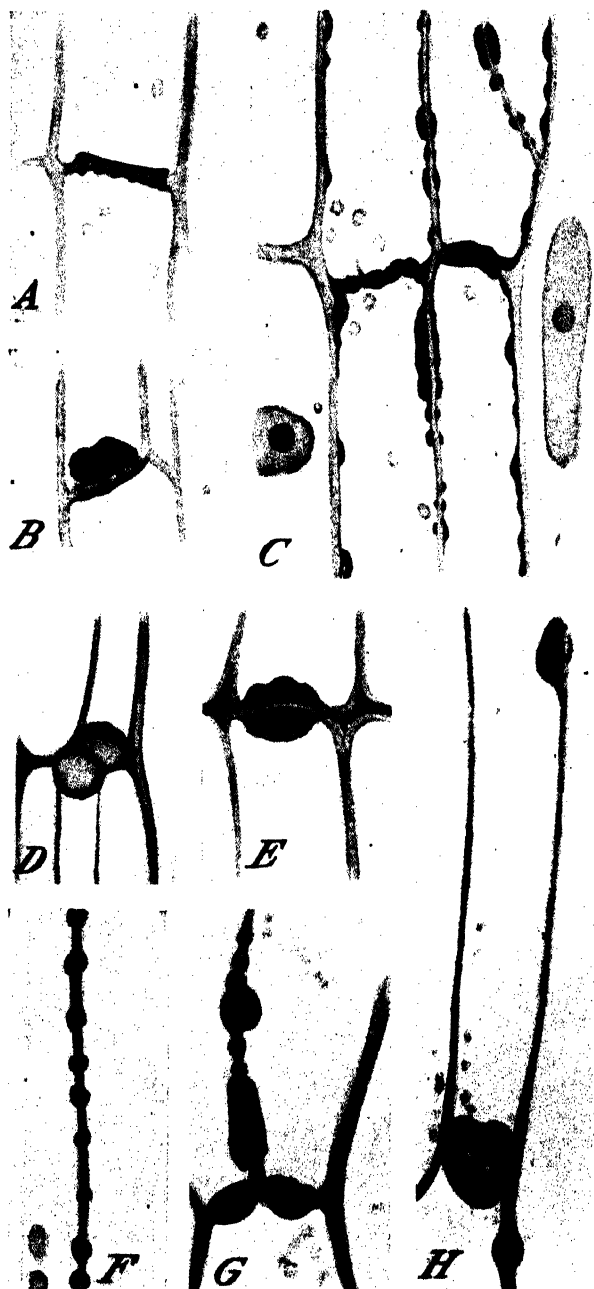
A, Phragmoplasts and early cell-wall formation in longitudinally dividing cell. × 1,450. B, Early stage in binucleated cell formation. × 1,450. C, Later stage in normal longitudinal division. × 1,400. D, Later stage in binucleated cell formation. × 1,450. E, Late anaphase preceding stage illustrated in B. × 1,350. F, Stage similar to D, except that the nuclei have the chromatin still in the form of contracted chromosomes. × 2,000. G, Two abnormal cell divisions in one cell row. × 1,000. H, Stage similar to B; the phragmoplasts form a circle, but the nuclei are not seen in this particular focus. × 1,350.

PLATE 8

A, Enlarged phloem parenchyma cell of taproot with dense vacuolate cytoplasm and large spherical nucleus undergoing chromatolysis. × 1,200. *B*, Hypertrophied nucleus with coarse flaky chromatin. × 1,400. *C*, Narrow phloem parenchyma cell with greatly elongated nucleus; the chromat in forms four pseudonuclei, each surrounded by a light halo. × 1,200. *D*, Hypertrophied nucleus with dense nuclear membrane and finely dispersed chromatin. Nucleoli are absent. × 1,400. *E*, Phloem parenchyma cell with large nucleus practically devoid of chromatin. There are many leucoplasts with starch. × 1,200. *F*, Greatly enlarged phloem parenchyma cell. The nucleus has only a little chromatin. Large inclusion body resembling chromatin extrusion in close proximity to the nucleus. × 1,500.

is the "cleavage nucleus", so called because of its resemblance to the mulberry fruit or the blastula stage of an embryo. Nuclei of this type are shown in plates 9 to 11. They are most commonly derived from the spherical or oval nucleus, but occasionally are related to the greatly elongated nuclear type. There appear to be two distinct classes of cleavage nuclei. To the first class belong those in which the cleavage furrows progress inwardly from the periphery. In their early developmental stages these differ from normal nuclei only in the somewhat scalloped appearance of the wall (pl. 11, *G*). As the invaginations deepen, the nucleus becomes divided into a number of segments which usually adhere to one another, so that the nucleus continues to retain its original size and shape. The segments into which the nucleus divides are usually of unequal size, the central segments being larger than the peripheral ones (pls. 9, *B, C, E, H;* 10, *I*). Occasionally segmentation is very symmetrical, so that the divided nucleus has a more or less honeycombed appearance (pls. 9, *F;* 11, *I*). In the nuclei of the second class, cleavage is entirely internal (pls. 8, *C;* 9, *G;* 10, *C*), so that in the initial stages such nuclei give the appearance of vacuolization, owing to poor fixation. As the cleavage progresses, the membranes of the individual segments are quite firm and distinct. The original nuclear membrane persists or may disintegrate, leaving the segmented inner region in the form of a mass of loosely adhering bubbles. Most of the nuclei as described and illustrated here are not distinct types, but rather transitory forms marking definite steps in the degenerative process.

CHROMATIN

The normal nucleus, according to the present-day concept (*17*), is composed of karyolymph and a reticulum of chromatic threads. The former composes the bulk of the nucleus and is in either the sol or the gel state. A division of the reticulum into chromatin and linin cannot easily be made objectively, since the existence of two distinct morphological substances is attributed by some workers to differences in degree of condensation of a single reticular substance; likewise, a division of the chromatin into oxychromatin and basichromatin may be related to the physical condition of the chromatin. Recent protoplasmic studies point more and more to the fact that nuclear substances are not chemically defined nucleoproteins but substances of changing composition, formed from a positive and a negative colloid.

Knowledge of the colloidal structure of the nuclear substances and their metabolism is still very insecure, and a distinction between normal and pathological is too often made subjectively. Ultramicroscopic studies show that even slight injuries suffice to cause flocculation of the chromatin (*18, p. 434*); chemical changes may cause irreversible gel formation, and frequently the close proximity of a parasite is sufficient to bring about such changes.

In beets infected with curly top the effect of the virus on the chromatin, as judged by its morphological structure, is manifold. The chromatin may appear flaky, coarsely granular (pls. 8, *B;* 11, *A*), finely divided, or entirely devoid of structure. In nuclei of the last-named type, the pronounced staining reaction shows the chromatin in a state of dissolution. Nuclei with finely divided chromatin (pls. 8, *D;* 9, *B, E*) frequently show clumping in localized regions; while in

some, distinct chromocenterlike masses are discernible (pl. 9, *A*).   In certain nuclei the chromatin has contracted into a central granular core, which is separated from the nuclear membrane by a more or less colorless zone.   Stained according to Feulgen, only the center gives a positive chromatin reaction, while the peripheral zone remains hyaline. In other nuclei of this type the central chromatin core is broken up into a number of irregular lumps, which are either located at the periphery or are scattered throughout the interior of the nucleus.   Finally, nuclei are found which contain only a few bright-staining granules of chromatin or inorganic protein crystalloids.

The chromatin of newly infected pericyclic nuclei with distinct hypertrophy is at first coarsely granular; later stages show regional clumping; and finally, under the influence of nuclease or of some other factor, the chromatin dissolves and may pass through the weakened and ruptured nuclear membrane (pl. 10, *G*) into the cytoplasm.

NUCLEOLUS

All nuclei of the root cells of sugar beet contain one or several nucleoli.   The latter are usually spherical and sometimes contain one or several vacuoles.   In diseased cells, the appearance of the nucleolus is quite variable and there is a certain correlation between chromatin and nucleolar behavior.   One notices extreme variation in nucleolar size and number in looking at hypertrophied nuclei at random.   There are nuclei, notably those with reduced chromatin content, which have a single nucleolus but of unusually large dimensions.   There are others where increase in nuclear substance is brought about through the formation of accessory nucleoli varying more or less in size (pl. 11, *F*).   Such numerical increase is often pronounced in greatly elongated nuclei and in those of extreme cleavage type (pl. 10, *H*).   Again, there are nuclei where the nucleolus is greatly reduced in size or from which it has disappeared entirely.   To this type belong mostly the hyperchromatic nuclei.   In general, it may be said that hyperchromasy is associated at the beginning with increase in nucleolar substance, which is followed by a gradual subsidence ending at times in the complete disappearance of the nucleolus.

This behavior of the nucleolus can best be studied in newly infected pericyclic cells of lateral rootlets.   The nucleoli of these cells show at first a definite hypertrophy followed by fragmentation (pl. 10, *A*, *D*).   The nucleolar fragments may sometimes gather at the periphery and during subsequent changes pass through the weakened or ruptured nucleolar membrane into the cytoplasm.

These pathological changes in the chromatin and in the nucleolar complex, as herein described, are more pronounced than those caused by other virus diseases, especially mosaic.   Certain early stages in the nuclear pathology of curly top have much in common with changes observed in host nuclei of plants affected with mycorrhizal fungi or plant and animal parasites (*18, pp. 212, 240*).   Such changes occur normally in the tentacle cells of *Drosera* after excitation (*18, p. 211*), but differ from pathological changes in being reversible, with the nucleus returning to normality after the subsidence of stimulation.

A, Nucleus with dense chromatin and distinct chromonemata; nuclear membrane faint. × 1,400. *B*, Greatly hypertrophied nucleus showing the beginning of peripheral cleavage. × 1,450. *C*, Nucleus similar to *B* with advanced peripheral cleavage. × 2,000. *D*, Nucleus from greatly enlarged cell; nuclear membrane thick and beaded; chromatin homogeneous, containing a few nucleolar fragments. × 1,500. *E*, Greatly hypertrophied nucleus in early stages of cleavage. × 1,450. *F*, Mulberry type of cleavage nucleus in narrow phloem parenchyma cell. × 1,450. *G*, Honeycomb type of cleavage nucleus. Segmentation is internal and does not involve the nuclear membrane. × 1,500. *H*, Extreme type of irregular cleavage. × 1,450. *I*, Similar to *H* but illustrating a different type. × 1,450.
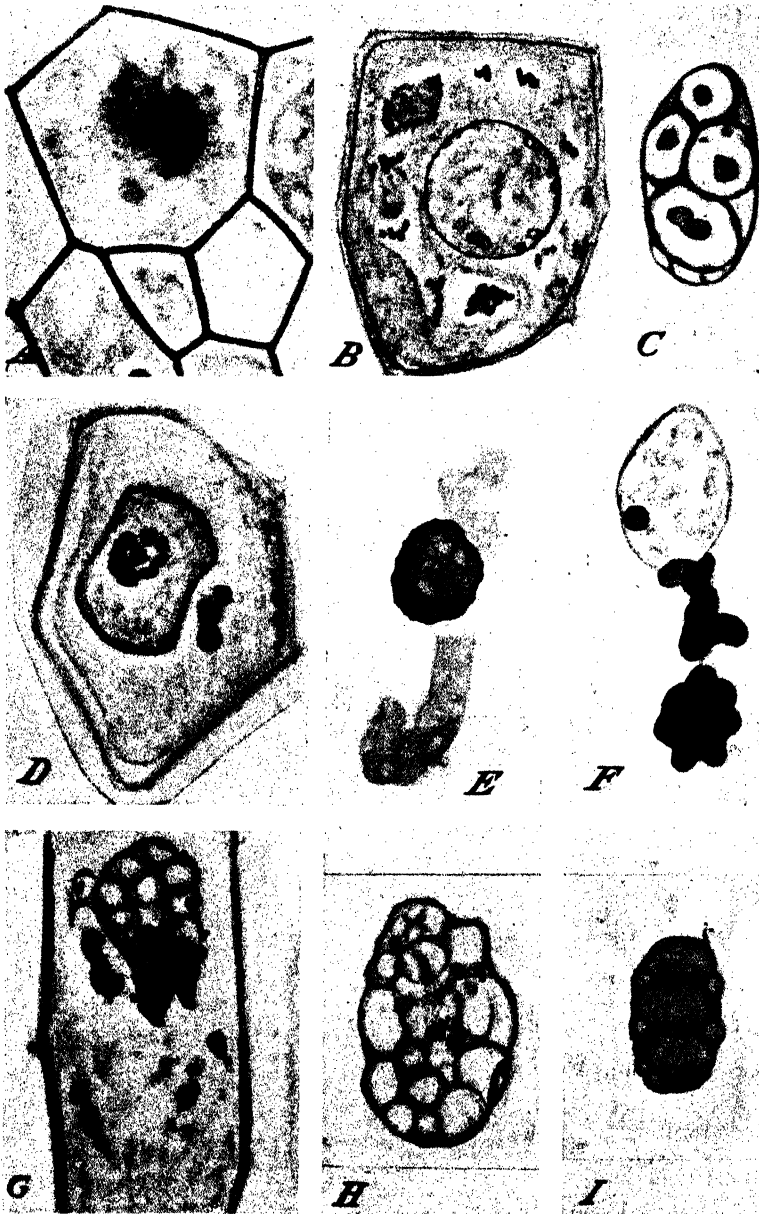
A, Hypertrophied pericyclic cell of young lateral rootlet with empty primary sieve tube at lower right-hand corner. The nucleus of the pericyclic cell has degenerated, the nucleoli are greatly enlarged, and the nuclear membrane has disappeared. × 3,000. *B,* Hypertrophied pericyclic cell with enlarged nucleus, fragmented nucleolus and inclusion bodies; the latter are of cytoplasmic origin and resemble division stages of some chytrid parasite. × 3,000. *C,* Peculiar type of cleavage nucleus. There is slight peripheral cleavage while the center is occupied by a number of globules, each with a chromatin center. × 2,000. *D,* Hypertrophied young pericyclic cell with typical nucleolar fragmentation. × 2,000. *E,* Enlarged nucleus and inclusion body in phloem parenchyma cell stained according to Feulgen. × 1,450. *F,* Enlarged nucleus and inclusion body stained in the ordinary way. The inclusion body takes a typical chromatin stain and gives the appearance of having been formed from chromatin diffused through the nuclear membrane. × 1,450. *G,* Nuclear degeneration and nucleolar fragmentation in pericyclic cell. × 2,000. *H,* Extreme type of cleavage nucleus. Distinct nuclear membrane absent; nucleoli numerous. × 2,000. *I,* Typical cleavage nucleus; nucleoli are wanting. × 1,400.
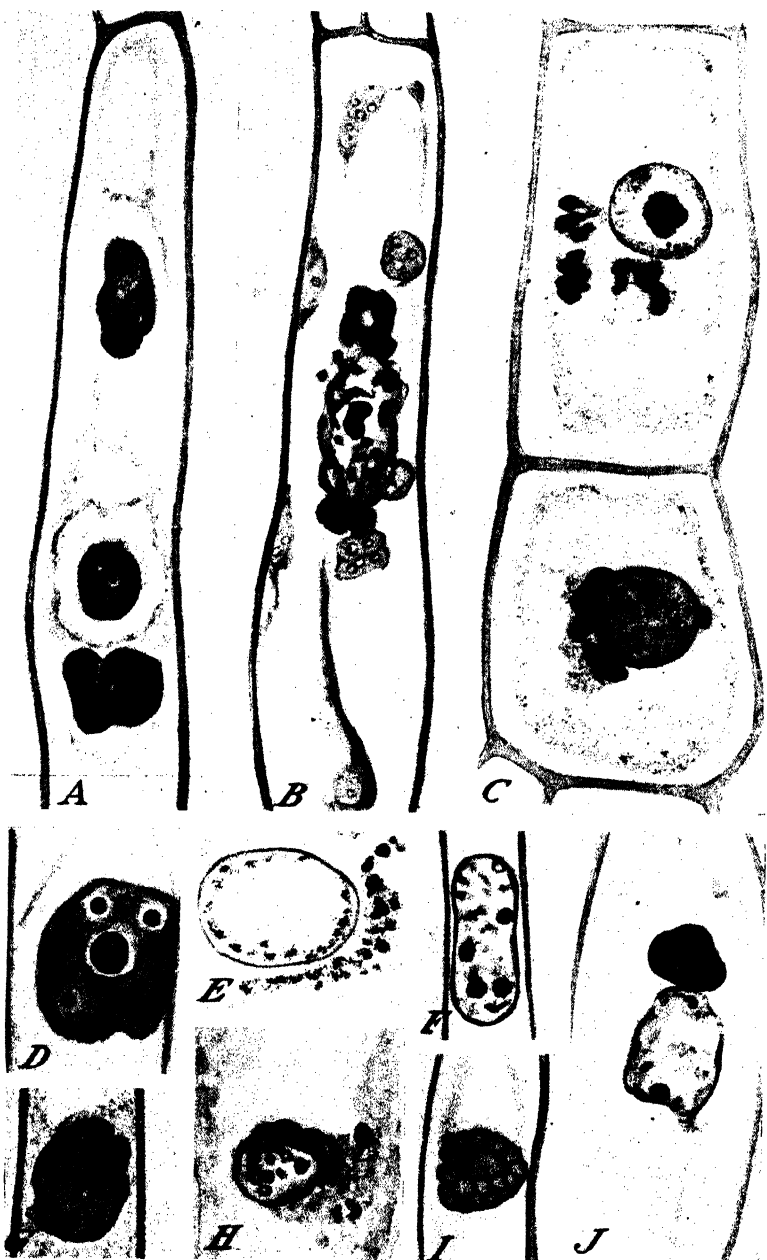
A, Hypertrophied phloem parenchyma cell from tap root of beet seedling. The chromatin of the nucleus is contracted into a central clump while at either side of the nucleus is a large compact inclusion body. × 1,500. *B*. Similar to *A*. The nucleus has a brightly stained nucleolus; the two inclusion bodies are in contact with the nucleus. There are a number of swollen starch-bearing leucoplasts. × 1,500. *C*, Similar to *A* and *B* except that the two cells are younger. The chromatin in the nucleus of the upper cell is contracted; the inclusion bodies are in the nature of partly discrete masses. The chromatin in the nucleus of the lower cell is finely dispersed, the inclusion bodies similar to those of upper cell. × 1,400. *D*, Fusion nucleus. × 1,500. *E*, Nucleus from an old hypertrophied phloem parenchyma cell. The nucleus contains little chromatin. The mass outside the nucleus is in part amorphous precipitate from an inclusion body, in part cytoplasm. × 1,500. *F*, Somewhat hypertrophied nucleus with a number of nucleoli. × 1,500. *G*, Initial stage of peripheral cleavage. × 1,500. *H*, Nucleolar fragmentation with nucleus in last stage of degeneration. × 1,500. *I*, Mulberry type of cleavage nucleus. × 1,500. *J*, Phloem parenchyma cell with nucleus and single compact inclusion body. × 1,550.

NUCLEAR MEMBRANE

Pathological disturbances in the nucleus frequently involve the nuclear membrane, as shown by its modified appearance and staining reaction. Such changes are not surprising if the nuclear membrane is viewed as a semipermeable layer at the nucleocytoplasmic interface, governing the transfer of substances between cytoplasm and chromatin. It is conceivable that the virus or a toxin associated with the virus, after permeating the cytoplasm, would induce, directly or indirectly, permeability changes in the nuclear membrane and subsequently initiate the far-reaching disturbances in chromatin and nucleolus described above; or the visible changes in the nuclear membrane could be considered a belated secondary phenomenon, since no effect on the structure of the membrane becomes evident until after the chromatin has been much damaged.

Changes in the structure of the nuclear membranes are progressive or regressive, and not reversible.

Progressive changes comprise a thickening of the nuclear membrane (pl. 9, *D*). The thickening is usually uniform but occasionally discontinuous, giving the nuclear wall a beaded appearance. Nuclei with membranes of either type are common to phloem parenchyma cells (pl. 8, *B*, *D*), though the beaded form is usually found in connection with chromatolytic degeneration. The thickening of the nuclear membrane is at times more apparent than real and is occasioned by the deposition of chromatin on its inner boundary. The beaded appearance of certain membranes is probably the result of unequal deposition of chromatin, as shown very strikingly in preparations stained according to Feulgen.

Regressive changes cause a gradual attenuation of the membrane, leading often to its complete disappearance. This type of degeneration is found mostly in newly affected pericyclic cells of lateral rootlets (pl. 12). Obliteration of the nuclear membrane is also observed in extreme forms of cleavage nuclei. The nuclear segments, instead of adhering, may scatter through the cytoplasm and give the cell a pseudomultinucleate appearance. A very faint nuclear membrane is occasionally observed in certain hypertrophied nuclei of phloem parenchyma cells (pl. 9, *A*), though, as a rule, the walls of such nuclei are abnormally thickened.

GENERAL PATHOLOGY

The various types of nuclear abnormalities recorded by investigators during the past decades are grouped by Tischler (*18*, *p.* 439) under the following headings: Karyorrhexis, pyknosis, chromatolysis, and karyocholosis. These groups are briefly characterized as follows:

(1) *Karyorrhexis, or normal old-age death.*—This is commonly observed in antipodal and tapetal nuclei, also in those of bacterial galls, and in tissues after exposure to X-rays. In such nuclei the chromatin complex condenses into clumps of varying dimensions which later fragment into smaller bodies and finally pass through the nuclear membrane into the cytoplasm, where they are absorbed.

(2) *Pyknosis.*—This is found only in relatively young cells, and the primary cause is thought to be overproduction of basic chromatin. The nucleus takes on a spiny appearance, gradually loses its turgidity, and grows smaller; while the chromatin, which at first showed a strong contraction, dissolves and diffuses through the nuclear membrane.

(3) *Chromatolysis.*—Like karyorrhexis, this is a phenomenon of old age and affects normally the nuclei of sieve tubes, vessels, and tracheids. It is characterized by the dissolution of the basic chromatin, often preceded by the flocculation of the latter. The dissolved chromatin passes through the nuclear membrane and may collect in drops on its outer boundary. If stained prior to its diffusion through the membrane, the content of such nuclei appears as a homogeneous dark mass.

(4) *Karyocholosis.*—This designates an abnormal increase in nucleolar substance, usually accompanied by a reduction in chromatin, and is caused by an inhibition of the nuclear metabolism.

The nuclei of beets infected with curly top, as previously described' do not fit exactly into any of the four groups mentioned above.

Most pronounced is the phenomenon of karyocholosis, which is always associated with some type of chromatin aberration. Thus, increase in nucleolar substance, whether brought about by the enlargement of a single nucleolus or by fragmentation and subsequent growth of the fragments, is observed in young and old nuclei, in nuclei affected with karyorrhexis or pyknosis, or in those undergoing chromatolytic dissolution.

Hypertrophied nuclei of phloem parenchyma or still undifferentiated cambium derivatives of diseased taproots most often show karyorrhexis. The nuclear membrane appears thickened, and the chromatin forms irregular lumps and is contracted into a central core or finely dispersed. The presence of compact or lobed aggregates in the vicinity of the nucleus is suggestive of chromatin emission, but the negative Feulgen reaction argues strongly against their nuclear origin.

The spiny nuclear forms characteristic of the later stages of pyknotic degeneration have not been observed, though the chromatin configurations typical of the early stages of this type of nuclear disorder are seen frequently. As previously stated, pyknosis is found in relatively young cells, such as are represented by the pericycle of newly infected lateral rootlets. The nuclei of these cells show at first a pronounced overproduction of chromatin and karyocholosis. Nuclear enlargement, however, as a rule is not followed by chromatin contraction and reduction in mass, but often ends with a rupture of the nuclear membrane and the subsequent intermixing of nucleoplasm and cytoplasm.

Chromatolysis as a separate symptom of degeneration is probably of rare occurrence, but it often accompanies karyorrhexis and pyknosis.

CYTOPLASMIC CHANGES

Cytoplasmic disturbances in cells affected with curly top are often less pronounced and, as a rule, less readily recognized than changes in the nucleus, although the cytoplasm comes under the influence of the parasite sooner than the chromatin. The reaction of the cytoplasm to virus infection is prompt and varied, controlled by the type of cell and the degree of its maturity. In the pericycle of young laterals which become infected through the diseased taproot, the cytoplasm of the affected cells is uniform in appearance; while in the taproot, where many of the cells have already reached a certain degree of differentiation, the pathological picture varies with each cell or group of cells. In other words, the nature and progress of pathological changes depend largely on the age at which the cell comes under the influence of the curly top virus.
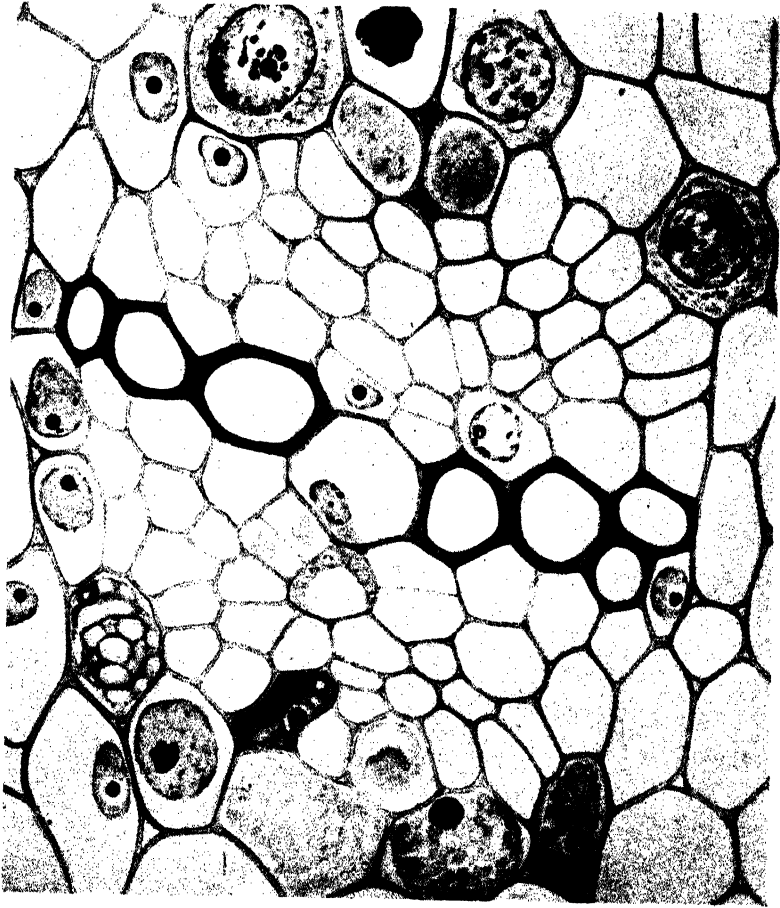
Cross section of diseased lateral rootlet about 7 days after inoculation. The primary protophloem sieve tubes are completely collapsed; the pericyclic cells are greatly hypertrophied and their nuclei are in various stages of degeneration. ✕ 1,600.
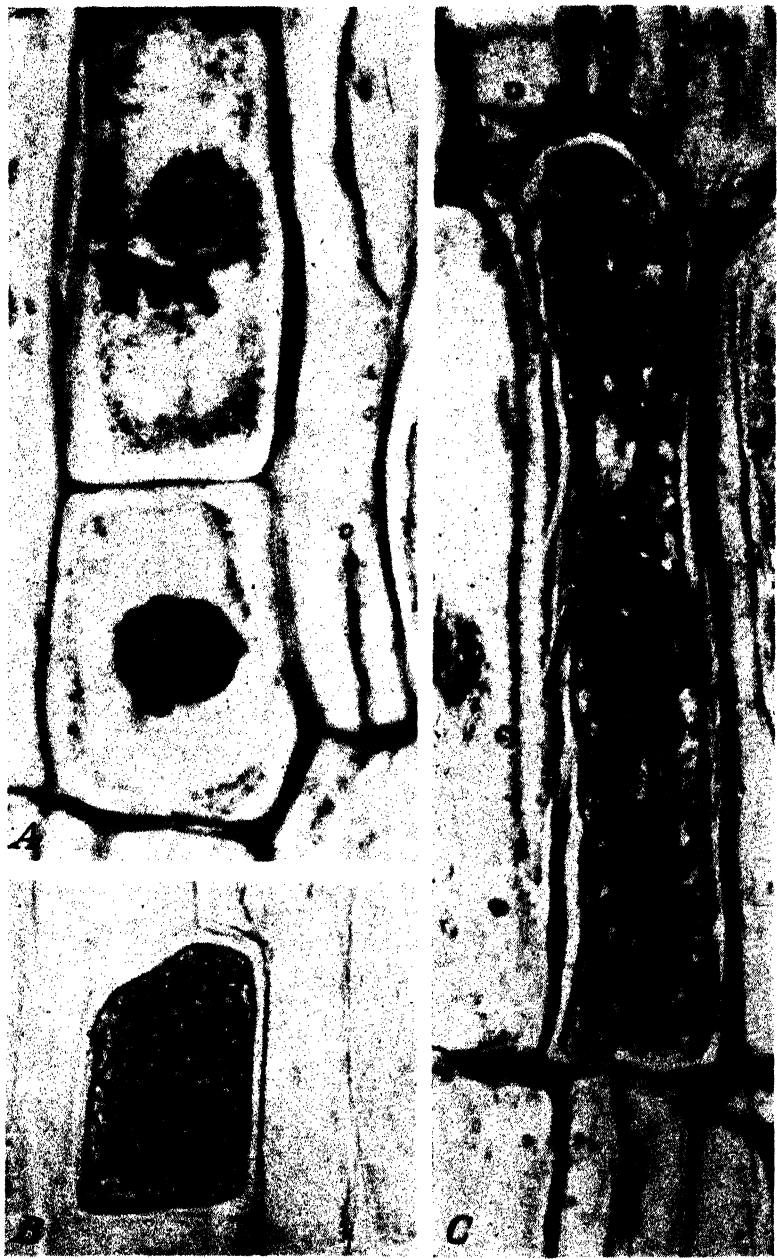
A, Two phloem parenchyma cells from a taproot of seedling, showing inclusion bodies.   B, Broad phloem parenchyma cell with dense cytoplasm and chromatin, surrounded by empty cells.   C, Phloem parenchyma cell from taproot seedling soon after infection, showing dense cytoplasm and chromatin. All ×
1,400.

Since cytoplasmic disturbances, whether microscopically recognizable or not, should precede any nuclear disorganization, the initial stages of such disturbances are to be expected in cells where the nucleus appears still more or less normal. In cells with pronounced nuclear pathology, the diseased cytoplasm, if there is any remaining, is no longer suitable for study. Likewise, a study of the cells that become affected during the later stages of their ontogeny, when the amount of cytoplasm is greatly diminished, is of value only so far as the nucleus is concerned. Such belated disturbances are probably seldom recognized, since it appears that only relatively undifferentiated cells suffer ill effects from the virus.

The bulk of the root tissue of young beet seedlings is composed of cambium cells and young phloem tissue, part of which is in the process of differentiation. For a study of cytoplasmic changes following curly top infection, the young phloem parenchyma cells are especially instructive. The sieve tubes are of little interest, since their cytoplasm is normally scanty and soon becomes limited to a thin parietal layer. More interesting are the hyperplastic cells, those sieve-tubelike elements which play such a prominent part in the pathological picture of the phloem in the aerial organs. They seldom form a solid tissue in infected roots but appear scattered, supplanting sieve-tube and phloem parenchyma in localized regions; they are probably the result of misdirected differentiation in certain cambium cells. The cytoplasm of these cells is fibrillar rather than granular and stains more deeply than that of the surrounding cells. At an early age in their ontogeny the hyperplastic cells possess slime bodies and sieve-tube plastids which are more prominent than those found in ordinary sieve tubes and which are slow to disintegrate, being still conspicuous at a time when the slime bodies of the sieve tubes have already disappeared.

During the early stages of infection the young phloem parenchyma cells contain, as a rule, an abundance of densely staining cytoplasm. The affected cells occur singly or in groups (pl. 13, *B*, *C*), indicating a certain preference on the part of the virus or, what is likely, the ability of certain cells to overcome infection. The fixed cytoplasm is structurally finely granular (pl. 8, *C*) or fibrillar with knoblike thickenings at the intersection points of the threads. The nuclei of these cells either are still normal or are undergoing progressive modifications due to the early stimulating effect of the virus. Simultaneous with the first cytoplasmic response is a gradual enlargement of the cell as a whole. The duration of this first or stimulative phase in the degeneration of the cytoplasm is hard to predict, being influenced by the reaction of the nucleus as well as by the environment, i. e., the normal or diseased condition of the surrounding cells. The cytoplasm may continue to increase in mass, but it frequently fails to keep pace with the growth of the cell. As the disease progresses, degenerative changes in the cytoplasm become more and more evident. The picture becomes still more confusing through the appearance of inclusion bodies and increased interference on the part of the nucleus, which, as already pointed out, often undergoes chromatolytic changes resulting in emission of nucleolar fragments and possibly other nuclear material into the cytoplasm. Eventually the cytoplasm of all hypertrophied cells is reduced, often so drastically as to leave the cells practically empty. At times, however, changes are of a different nature, when

the cytoplasm and its inclusions become rapidly transformed into a homogeneous red-staining substance filling part or all of the cell lumen.

CELL INCLUSIONS

The phloem parenchyma cells of beet roots contain leucoplasts as normal cytoplasmic inclusions. Their number is small but may materially increase (pl. 8, *E*) following curly top infection. As the disease advances, the leucoplasts often degenerate, forming, together with the crumbling protoplasmic structure, those gummy cell occlusions which are among the characteristic features of the last stages in curly top degeneration.

Calcium oxalate is of common occurrence in the aerial and underground organs of the sugar beet. In the taproot, it is in the nature of crystal sand, which is limited in its distribution to a few phloem and interzonal parenchyma cells. In diseased roots, the number of calcium oxalate cells increases greatly, often so much as to impart a silvery sheen to a longitudinal section viewed under low power. The crystal-bearing cells are devoid of cytoplasm and nucleus and normally remain unstained, but occasionally they color distinctly. An explanation for this phenomenon might be sought in a possible modification of the organic substrate of the crystalline deposit, resulting in absorption of the stain.

There remains to be considered a rather important group of abnormal ergastic structures generally known as inclusion bodies.

These bodies, first noted in sugar beets by Rawlins (*15*), were recently described and illustrated by Esau (*11*), who confirmed the observations of Rawlins but modified them by putting the X- and Y-bodies into one class. The inclusion bodies, according to Esau, are first observed in young cells in close proximity to the protophloem sieve tubes. They are seen as small dark bodies, which subsequently enlarge, ranging in appearance from irregularly lobed aggregates to more or less round compact structures. The compact form is of short duration, as it soon breaks up into a conglomerate of dark globules embedded in a light-staining substrate. The disintegrating body appears to be contained within a vacuole, though no distinct membrane is discernible. The large compact bodies occur singly, in pairs, or in irregular dark clumps. Although not commonly associated with the nucleus, they are occasionally found in such close proximity to the latter as to deform it. In staining reaction and in structure, the compact bodies resemble nuclear material, but they are not of nuclear origin.

Since the investigations of Esau and those recorded in this paper are based on similar material, it is only natural that there is a certain agreement in the description of the anatomical pictures. There is, however, also a complementary value in these two records because of the difference in interpretation of apparently similar phenomena, resulting from the use of different stains, especially the Feulgen chromatin reaction, and from the subjective preference in viewpoint.

The different types of inclusion bodies may be divided roughly into two groups: Those which form irregularly lobed aggregates (pls. 8, *F*; 10, *F*; 11, *C*; 13, *A*) and those forming round compact bodies. The inclusions of the first group vary in size from isolated oval bodies to large connected or discrete masses, which at times fill a considerable part

of the cell lumen. Their relative position in the cell is indefinite and their orientation in regard to the nucleus follows no rule. The large compact bodies of the second class occur singly (pl. 11, *J*) or in pairs (pl. 11, *A*, *B*) and are then in juxtaposition with the nucleus. The inclusion bodies of the taproot may be found in any of the undifferentiated cambium derivatives which in a young seedling form the major part of the root tissue. The cells which contain them usually show signs of incipient degeneration. Such cells are usually enlarged and may contain an abundance of lininlike cytoplasm, but, as a rule, the amount of cytoplasm is greatly diminished. The nucleus is also enlarged, and the chromatin is finely granular or in irregular lumps which at times are contracted into a central core. Nucleoli are often wanting or, if present, no longer react to the safranine stain. Leucoplasts are usually present, often in large numbers, and are swollen and frequently chemically changed.

Structurally the inclusion bodies are very homogeneous, with no trace of granulation. Vacuoles are faint but relatively more prominent in Feulgen preparations (pl. 10, *E*). The staining reaction of the inclusions to Flemming's triple stain is like that of the chromatin retained in the nucleus, usually dark lavender; but in preparations stained according to Feulgen, this similarity disappears. It is at first rather difficult to locate these bodies in a Feulgen preparation, unless the condenser of the microscope is lowered; and even then their outline is quite faint

Esau's speculations concerning the ontogeny of the inclusion bodies are problematical. Although it seems safe to assert that the small rounded bodies are first seen in cells which by the appearance of cytoplasm and nuclei suggest recent infection, the larger and apparently older bodies occur in practically any type of affected cells, regardless of size and content. There is little to suggest an evolution from the discrete and loose aggregates to the large compact type. On the other hand, it appears to be true that occasionally compact inclusions break up, filling what appear to be large vacuoles with their debris. Although the inclusion bodies often appear shortly after infection and may assume prominence after the cells show the effect of cytoplasmic and nuclear degeneration, the duration of their existence is hard to determine. Since they are found just as abundantly as other types and since they are prominent in young and old cells alike, it would seem that the compact stage is not necessarily passed through rapidly. In judging the age of the inclusion bodies by the general appearance of the affected cells and their nuclei, it must be remembered that degenerative cell changes may at times be very rapid and that such changes are related to the initial infection and to the degree of maturity of the affected cell.

Judged by their differential staining pattern with Flemming's triple stain, the inclusion bodies strongly suggest a nuclear origin. Esau (*11, p. 420*) also speaks about their similarity in appearance to masses of chromosomes, and Tischler's figure (*18, p. 444, fig. 244*) could easily find a counterpart in many of the microscopic pictures from infected beet tissue. However, there is much variation in the appearance of nuclei that contain inclusions. While some of the enlarged nuclei are practically empty, others have a superabundance of chromatin. The negative Feulgen reaction speaks strongly against their chromatin nature, but it could be argued that the chromatin,

prior to emission, was so greatly altered that the Feulgen reaction was no longer positive; in that case, the chromatin retained in the nucleus should also be colored only faintly, which, however, is not the case. Occasionally, however, nuclei are associated with large inclusion bodies in which most of the chromatin retained in the nucleus stains very faintly or not at all.

It is equally difficult to link the formation of the inclusion bodies to local changes in the cytoplasm, at least in newly infected cells which are hypercytoplasmic. It is conceivable, however, that local proteolysis in the cytoplasm plays an active part in the formation of inclusions in cells where changes are so rapid that the entire content becomes metamorphosed into a multicolored mass, filling all or the greater part of the cell lumen.

Since a nuclear or cytoplasmic origin of many of the inclusion bodies is problematical, we must consider most of them byproducts, however specialized, of the pathological metabolism of the infected cells. As such, they would be homologous with similar bodies associated with mosaic and other virus diseases by numerous investigators since the days of Iwanowski.

Of more than passing interest are the inclusion bodies in affected pericyclic cells. Here the struggle between the virus or its toxin and the host cell is more swift and deadly than in the immature cambium derivatives and in the phloem parenchyma of the taproot, and the ever-changing microscopic pictures which mark the contest succeed each other quickly and are often difficult to interpret. The stimulative phase, leading to general hypertrophy, hyperchromasy, and karyocholosis, soon gives way to the destructive phase, characterized by nucleolar fragmentation and dissolution phenomena in chromatin and cytoplasm. The nucleolar fragments escape into the cytoplasm, where they are recognized by their staining reaction. However, not all of the red-staining inclusions are nucleolar in origin; some, such as those shown in plate 10, *G*, are due to local changes in the cytoplasm. Quite often these local changes that lead to the formation of pseudonucleolar inclusion bodies are so general that a major part of the cytoplasm stains more or less intensely with the safranine component of the triple stain. Often the diseased pericyclic cells contain as transitory accompaniments aggregates of chromatin and inclusion bodies, but these structures are not as clearly delimited as those described for the phloem parenchyma of the taproot.

Mention should also be made of peculiar intensely blue-staining cytoplasmic inclusions which in their shape resemble individual raphide crystals. They are of frequent occurrence in newly infected cells and are not unlike certain bodies described by Clinch (*5*) for some solanaceous plants.

DISCUSSION

The following discussion of the results of this investigation aims to establish the sequence of events by interpreting the anatomical pictures of root tissue of curly top plants and to evaluate the underlying causes for selective cellular injury through an appraisal of the normal potencies of such cells.

In lateral roots, with their limited amount of stelar tissue, the cells most easily affected are those of the pericycle, a single-layered tissue just inside the endodermis. In certain regions of the stele, pericyclic

cells abut externally and laterally on the virus-bearing primary sieve tubes (pl. 4, *A*), while centripetally to the latter is undifferentiated procambium.   Since the chances for coming under the influence of the virus are equal and yet a pronounced susceptibility is shown only by the cells of the pericycle, an inquiry into its properties becomes desirable in order to help explain the differential resistance to the virus.   Structurally the pericycle of roots is an unusual tissue, giving rise, as the need may be, to meristems, which form lateral roots, phellogens, and secondary cambiums, as well as adventitious roots or stems.   The cells of such a tissue would naturally be very reactive, and their nuclei and cytoplasm would be most labile and responsive to stimuli even under normal conditions.   In the presence of specific foreign irritants, such as a virus or toxins, one could expect far-reaching changes in the cellular metabolism that would eventually cause irreversible alterations in the nucleus and finally death.   That this actually happens has been shown in the present paper.

Cell response to primary infection in the taproot does not deviate fundamentally from that in laterals, if one takes into account the differences in the structure of these two organs.   The young taproot at time of infection possesses one or two supernumerary cambiums, each with several rows of immature derivatives.   Because of this anomalous structure, the lateral spread of the primary infection is not uniformly centripetal or centrifugal but sporadic, depending on the distribution of the sieve tubes in the immature supernumerary rings.

The cells of the seedling taproot, which are the first to become diseased in the course of primary infection, are immature cambium derivatives, lineal descendants of the original pericycle and primary phloem parenchyma.   At the time of exposure to the virus, most of these cells are little specialized, the protoplasm being somewhat less labile and more resistant to stimuli than that of the pericycle of lateral rootlets.   This is evident from the following facts: The time required for abnormalities to appear is extended beyond that observed for the pericycle; the struggle between host protoplasm and virus is prolonged; the reactions are more elastic, not always terminating in early death but leading to the development of unusual cellular hypertrophies and nuclear monstrosities, causing frequently such deviations from normal development as the creation of cell types intermediate between sieve tubes and phloem parenchyma.

It appears that early primary infection in both taproot and laterals is dependent on relatively meristematic totipotent cells with labile protoplasm which reacts readily to stimuli.   Such prerequisites are possessed by cells of the pericycle and the undifferentiated cambium derivatives.

The sequence of events in the cellular pathology of beets infected with curly top is normally easily followed in the case of lateral rootlets, in which the changes occur chiefly in the pericycle.   But in the complicated structure of the taproot, with various types of cambium derivatives, each having its own ontogeny, analysis becomes difficult.

The first microscopically recognizable changes in the pericycle of the rootlet are noticed about 3 days after inoculation, while according to Esau (*11*, *p. 405*), initial disturbances in the leaf may show as early as the second day after insect feeding.   The pathological pictures indicate the presence of two more or less distinct phases: An

anabolic phase, leading to general hypertrophy, and a catabolic or destructive phase, terminating with the death of the cell. The threshold of the catabolic phase finds the affected cells greatly enlarged, the nuclei hyperchromatic and the nucleoli often fragmented; the staining reaction of chromatin and cytoplasm is usually pronounced. In the course of these developments the structure of the chromatin often becomes alveolar or coarsely reticulate, the wide meshes suggesting internal cleavage (pl. 10, *G*). Destructive changes may set in while hypertrophy is still in progress and are indicated by dissolution phenomena in the nucleus and proteolysis in the cytoplasm. Simultaneously or soon afterward, nucleolar fragments and altered bits of chromatin pass through the weakened or partially destroyed nuclear membrane into the cytoplasm. Here they mingle freely with cytoplasmic fragments (pl. 10, *G*) altered by local proteolysis, and, because of similar staining reaction, soon lose their visible identity. More or less indefinite inclusion bodies assume temporary prominence in the pathological picture. They may disappear entirely or become transformed into amorphous precipitates and, when catabolic changes are very rapid, they form an integral part of the red-staining occlusions which fill the entire cell lumen.

The pathological picture of the diseased taproot is complicated because of the diverse nature of the cambium derivatives, and in analyzing the various cellular and nuclear anomalies it is essential not to confound parallelism with developmental succession.

In healthy beets, the cambium derivatives mature into sieve tubes, companion cells, and phloem parenchyma; but in diseased individuals there are, in addition, elements that are intermediate in structure between sieve tubes and phloem parenchyma. These pseudosieve tubes are absent from early primary infection but become increasingly conspicuous after the disease is well established. They are readily recognized by their numerous slime bodies and sieve-tube plastids. The accompanying cell-wall changes are very striking and have been described in detail herein, under "Pseudocallus."

The first microscopic changes in the taproot are limited to those cambium derivatives which mature into phloem parenchyma. Since their number is relatively small, moreover, and since they are less susceptible than pericyclic cells, the pathological picture of a young taproot 4 to 6 days after infection does not depart significantly from the normal structure. As a rule the cellular and nuclear changes are slow, though at times there is an almost instantaneous metamorphosis of the cell content into a gummy cell occlusion, which explains the sporadic appearance of severely diseased cells in otherwise normal tissue. Cytoplasmic and nuclear changes agree in principle with those described for the pericycle, except that the changes are less conspicuous and do not always lead to the destruction of the cell content but rather to the development of abnormal nuclear forms and transitory inclusion bodies.

The different types of nuclear anomalies have been described herein; it will suffice to call attention once more to the fact that they are not distinct forms but transition stages of certain types, of which the cleavage nucleus is the most conspicuous. The impetus for the development of the different types is probably given at the time of infection and is related to the degree of cellular susceptibility and the

quantity of the initial stimulus. This, however, does not preclude the fact that subsequent changes in the environmental conditions of the affected cell might change the normal course of nuclear pathology. Great susceptibility, coupled with maximum initial stimulation, probably results in more pronounced nuclear anomalies than does belated infection, although the actual proof would be difficult to produce. The ontogeny of a typical cleavage nucleus usually has its beginning in superficial imaginations which soon become cleavage furrows, dividing the content into asymmetrical pieces. Since cleavage nuclei are not observed until about 8 days after infection and since at the time of initial invagination the nuclei are already hypertrophied and the nucleoli often fragmented, cleavage is, in most cases, a secondary phenomenon.

The changes in the nucleocytoplasmic relationship following infection depend on initial stimulation, age, and the relation of the affected cell to its immediate environment. Whether the first effect is on the cytoplasm or on the chromatin cannot be judged objectively, since there are no known methods fine enough to serve as indicators for such early changes; however, it is undoubtedly true that even very slight changes will tend to upset the finely adjusted balance in the protoplasmic structure. Changes in the nucleus, however slight, must needs be felt in the cytoplasm; and changes in the latter will tend to further upset the normal order, until the cumulative effect of such stimulation prevents reversibility and leads to the death of the cell. The formation of aberrant cytoplasmic inclusions and the emission of nucleolar fragments and possibly of much altered chromatin are closely related to the cellular metabolism, with the time factor playing an important and varied role.

SUMMARY

Judged by its effect on the inner root structure, curly top of sugar beet is primarily a nucleocytoplasmic disorder, but its study is of interest also because of the peculiar influence of the virus on the ontogeny and structure of certain cells of the stelar tissue.

Early primary disturbances are limited to the pericycle and the immature cambium derivatives, in other words, to cells which are multipotent and possess labile protoplasm, normally very responsive to stimuli.

Under the influence of the virus, the affected cells and their nuclei hypertrophy. In this process, the nuclei may expand symmetrically, become irregular, or assume odd shapes, as exemplified by the "cleavage nucleus." Many of these abnormal nuclear forms are transitory stages of certain basic patterns, more or less predetermined at time of infection but often modified through secondary stimulation.

The changes in the affected cells are marked by two phases. These usually overlap and their relative duration appears to be dependent on the age of the cell at the time of infection, its immediate environment, and the degree of initial stimulation. During the first or anabolic phase, there is an increase in nucleolar material and chromatin, accompanied by changes in the morphological structure of the latter. The threshold of the second or catabolic phase marks the beginning of irreversible changes in the nucleus, which are often characterized by dissolution phenomena and the possible emission of

altered chromatin into the cytoplasm. Nucleolar fragments also find their way into the cytoplasm and remain there unaltered, but often lose their visible identity because of proteolytic changes in the latter.

Cytoplasmic changes usually parallel those in the nucleus during the early part of the disease; later stages are marked either by mere quantitative reduction or by disorganization due to local or general proteolysis.

There are two types of cytoplasmic inclusions. To the first group belong calcium oxalate and leucoplasts; they are normally found in healthy plants but often increase enormously in diseased roots. To the second group belong structures foreign to healthy cells. Prominent, though transitory in nature, are nucleolar fragments and possibly chromatin extrusions with the chromatin in a greatly altered state, staining only very faintly by the Feulgen method. Amorphous precipitates that show great variation in staining reaction are also very common. Their origin is not always clear but can be attributed to the disintegration of certain compact inclusion bodies and to local proteolysis of the cytoplasm.

As a result of what appears to be misdirected differentiation in the maturation of cambium derivatives, there are formed hyper plastic cells, sieve-tubelike elements with plastids and slime bodies but without sieve plates. The walls of the cells become covered with pseudocallus.

The cells of the supernumerary cambiums and their derivatives divide longitudinally after the method of cambium cells first described by Bailey (2) for the white pine. Incomplete longitudinal divisions, resulting in binucleate and multinucleate cells, are quite common.

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RELATIVE SUSCEPTIBILITY OF CERTAIN SPECIES OF GRAMINEAE TO CERCOSPORELLA HERPOTRICHOIDES [1]

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INTRODUCTION

Cercosporella herpotrichoides Fron, which causes an important foot rot of winter cereals in the Pacific Northwest and in northwestern continental Europe, has been known to mycologists since 1912 (*6*),[2] but its importance as a parasite of winter cereals was determined much more recently (*2, 3, 4, 5, 13, 16*). The published information on the host range of *C. herpotrichoides* is largely confined to brief reports on the relative susceptibility of cultivated varieties of winter wheat (*Triticum aestivum* L.) and winter barley (*Hordeum vulgare* L.). Sprague and Fellows (*16*) and Sprague [3] have shown that certain types of wheat are more severely attacked in the field than others and that most varieties of barley are more resistant to the fungus than is wheat but less so than oats (*Avena sativa* L.) and certain other grains. Schaffnit (*12*) reported that wheat and barley are susceptible to *C. herpotrichoides*, and he found definite correlation between the prevalence of "straw breaking" and the development of the sclerenchymatous structure of the plant. Moritz and Brockmann (*10*), who were apparently the first to recognize the disease in Germany, confined their study of *C. herpotrichoides* to wheat. Foëx and Rosella (*4*) listed the fungus as parasitic on all of a large number of wheat varieties that were tested, on two-row, "four-row", and six-row barleys, on several species of *Avena*, and on Broekema rye. Recently Foëx (*1*) reported that *C. herpotrichoides* infected nine species of *Triticum* and six species of *Hordeum*, and *Avena orientalis* only slightly. Nielsen (*11*) reported that *C. herpotrichoides* had been found on wheat in Denmark.

Data are presented on the relative susceptibility of a number of wild and cultivated gramineous hosts to *Cercosporella herpotrichoides* and on the results of experimental studies in the greenhouse at Corvallis, Oreg., and at the Arlington Experiment Farm, Arlington, Va. near Washington, D. C. The latter indicate that many of the field-grown, so-called resistant cereals are actually not resistant but escape only by virtue of certain timely climatological factors. Evidence also is presented of the possible source of the disease from the original sod-grass prairie sections of the Columbia Basin.

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NATURAL HOST RANGE

In the United States the cercosporella foot rot is almost entirely confined to sod-grass prairies whereon a certain specific native vegetation originally occurred (*14*). Sprague and Fellows (*16*) recently reported that in the Pacific Northwest *Cercosporella herpotrichoides* had not been isolated from any range or pasture grass, although lesions resembling those caused by it on cereals were found on *Agropyron inerme* (Scribn. and Smith) Rydb. The original collection on this grass was made in June 1929 at the margin of a field of winter wheat (Hybrid 128) in the Spokane River Valley of Washington. Since that date further collections of this type of injury on this grass were made in June 1931, 1932, and 1933 at the same general location. In 1933, typical cercosporella lesions were also found on *A. riparium* Scribn. and Smith, *Koeleria cristata* (L.) Pers., and *Poa secunda* Presl. (*P. sandbergii* Vasey) in the Spokane Valley. Pure cultures of *C. herpotrichoides* were obtained in 1933 from all of these species of grasses except *P. secunda*, but with considerable difficulty, as the faster growing *Wojnowicia graminis* (McAlp.) Sacc. and D. Sacc. was predominant in the lesions on the hosts at this season (*15*). During the same month typical eyespot lesions of cercosporella were found on the culms of *Bromus tectorum* L., which occurred as a weed in cercosporella-infested wheat fields on Peone Prairie, Spokane County, Wash. Pure-culture isolations of *C. herpotrichoides* were easily obtained from this host. This same grass was found affected at Friend, Wasco County, Oreg., and in Union County, Oreg., in 1934.

In June 1934, following an exceptionally mild, almost frost-free winter, typical cercosporella lesions were found on *Bromus inermis* Leyss., *B. marginatus* Nees, and *Sitanion hystrix* (Nutt.) J. G. Smith near Nezperce, Idaho. These grasses were growing at the edge of fields of winter wheat heavily infested with *Cercosporella herpotrichoides*.

As reported in a check list of diseases on Oregon Gramineae,[4] *Cercosporella herpotrichoides* attacked, in the field, *Aegilops cylindrica* Host, *A. ovata* L., *A. triuncialis* L., *Bromus tectorum* L., winter oats (*Avena sativa*), rye, wheat, einkorn, spelt, and emmer.

Those mentioned above represent the only collections of *Cercosporella herpotrichoides* made on naturally occurring range and pasture grasses in the United States during 7 years of intensive search.

EXPERIMENTS IN NATURALLY INFESTED FIELDS

CEREALS

A brief summary of the field studies conducted in the Pacific Northwest with respect to the susceptibility of wheat and barley varieties to *Cercosporella herpotrichoides* has been published (*16*). This report stated that "the relative susceptibility of varieties varies somewhat from year to year." This point was clearly brought out during the long growing season of 1933–34 in the Columbia Basin, when certain varieties that were previously somewhat "resistant" became virtually as susceptible as the average susceptible varieties.

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[4] SPRAGUE, R. A PRELIMINARY CHECK LIST OF THE PARASITIC FUNGI ON CEREALS AND OTHER GRASSES IN OREGON. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 19: 156–186. 1935. [Mimeographed.]

In order to facilitate comparison of the susceptibility of cereals grown in the field with those grown in the greenhouse, the relative susceptibility of a number of representative varieties is given in table 1 for the period from 1930 to 1935. The results will be considered later under the heading "Discussion."

TABLE 1.—*Disease reaction of certain varieties of cereals to Cercosporella herpotrichoides for the period 1930–35*

[Hybrid 128 rated as 100]

Host (species and variety)	C. I. no.	Reaction to *Cercosporella herpotrichoides*						
		1930	1931	1932	1933	1934	1935	Average
Avena sativa L. (oats):								
Winter Turf (Gray Winter)	1570	2	4	2	4	15	4	5
Hordeum vulgare L. (barley):								
Alaska	4106	95	100	95	85	105	90	95
Tennessee Winter	257	90	83					87
Winter Club	592	50	48	50	50	74	65	57
Olympia	6107					72	70	71
Secale cereale L. (rye):								
Oregon Winter		5	5	8	5	10		6
Triticum dicoccum Schrank (emmer):								
Black Winter	2357	30	29					29
Triticum durum Desf. (durum):								
Akrona	6881	101						
Kubanka	1440	103	101					
Triticum monococcum L. (einkorn)	2433	9						
Triticum spelta L. (spelt):								
Alstroum	1773		140	130				135
Triticum aestivum L. (common wheat):								
Federation	4734	80	78	90	88	95	90	87
Goldcoin	4156	105	115	113	110	115	115	112
Hohenheimer	11458	65	63	68				65
Hosar	10067	75	74	80				76
Kanred×Marquis	8245		97	98	140	80	105	104
Kharkof	8249	110	120	110	115	120	120	116
Ridit	6703	100	93	103	100	95		98
Turkey	4429	110	120	107	120	120	115	115
Wilhelmina (Holland)	11389	85	68	65		90-110	90	81
Triticum compactum Host (club wheat):								
Hybrid 63	4510	110	111	107	100	105	100	105
Hybrid 128	4512	100	100	100	100	100	100	100
Hybrid 143	4160	95	97	105	95	110	98	100
Triticum aestivum×Secale cereale (wheat×rye):								
Meister's amphidiploid	11403				T	24		

[1] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

OTHER GRASSES

One point of increasing importance in connection with any program for control of the cercosporella foot rot is the need for information on the relative susceptibility of various range-land or pasture grasses to *Cercosporella herpotrichoides*. Recent developments in connection with the adjustment of the acreage sown to wheat, together with a growing conviction that other crops must be rotated with wheat on cercosporella-infested soil, have increased the interest in the growing of grasses in these prairie sections. This has led to inquiries as to the susceptibility of various grasses to *C. herpotrichoides*, and although all field observations indicated that the sparsely seeded grasses would not become injured to any extent by the fungus, certain field trials were conducted to obtain definite information on this point.

A number of grasses were seeded in the regular cereal test plot in 16-foot rows spaced 1 foot apart on September 25, 1933, in cerco-sporella-infested soil at High Prairie, Wash. On February 5, 1934,

healthy mature plants of crested wheatgrass, *Agropyron cristatum*
(L.) Beauv., were obtained from D. E. Stephens, Sherman County
Branch Station, Moro, Oreg., and transplanted into cercosporella-
infested soil at High Prairie. Some of these were planted in the
infested wheat and others a short distance away in an unseeded plot.
Winter wheat was also transplanted from Moro to the unseeded plot
at the same time. The results of this experiment (table 2) indicate
that these grasses, which are necessarily sown in widely spaced rows or
thinly broadcast in the semiarid cercosporella region, will not become
heavily infected with cercosporella foot rot. It is shown in table 2

TABLE 2.—*Disease reaction of certain Gramineae to Cercosporella herpotrichoides
at High Prairie, Wash.*

[Records taken June 6, 1934]

| Host (species and variety) | Method of propagation | Culms | | Estimated loss |
		Height	Number	
		Inches		*Percent*
Arrhenatherum elatius (L.) Mert. and Koch.	Seeded in cereal plot	16	120	0
Agropyron cristatum	Transplanted to cereal plot	16	42	5.3
Do	Transplanted to unseeded plot	16	60	0
Bromus secalinus L	Seeded in cereal plot	10	151	0
Festuca elatior L	do	3	75	0
Festuca rubra L	do	3	137	0
Hordeum vulgare (Winter Club)	do	34	81	40.2
Triticum compactum (Hybrid 128)	do	33	181	68.3
Do	Transplanted to cereal plot	29	165	22.3
Do	Transplanted to unseeded plot	28	179	2.5

that crested wheatgrass, while susceptible when grown under favorable
conditions of humidity among the wheat plants, was healthy when
grown in the open. This work was done during the most favorable
season on record for the foot rot. It has been observed many times
and repeatedly proved that even the most susceptible varieties of
wheat may be kept almost free of foot rot by widely spaced seeding.

EXPERIMENTS IN THE GREENHOUSE

METHOD OF PROCEDURE

Most of the studies of *Cercosporella herpotrichoides* were conducted
at Corvallis, Oreg., during the cooler months (November to April) in
an unheated greenhouse maintained at a temperature ranging mostly
between 5° and 18° C. The relative humidity was maintained above
85 percent for the most part, as any prolonged period with humidity
below 70 percent distinctly retards the parasitic activity of *C.
herpotrichoides*.

Some studies were made at the Arlington Experiment Farm,
Arlington, Va., in a greenhouse in which the temperature was similar
to that in the greenhouse at Corvallis. Usually the relative humidity
was kept above 95 percent by the use of a mist spray. The plants at
Arlington Farm were grown under 250-watt lights from 4 p. m. until
midnight during each 24-hour period.

Plants were grown in somewhat infertile, unsterilized, cercosporella-free Newberg sandy loam at Corvallis or in a mixture of two-thirds unsterilized river sand and one-third loam at Arlington Farm. The inoculum consisted of active cultures of *Cercosporella herpotrichoides* grown on boiled and quadruple-sterilized wheat kernels in liter or half-liter Erlenmeyer flasks. In the majority of the artificial inoculation experiments from 1929 to 1933 the inoculum was added to the soil at seeding time. The plants were grown either in cubic-foot boxes or in benches having a soil depth of 8 inches. The inoculum was placed above the seed with one-half inch of soil intervening and was applied at the rate of four to six of the fungus-covered grains of inoculum to each cereal seed or large grass seed sown. This method of inoculation induced an extremely severe foot rot with abnormally pronounced stunting of infected culms. By using this method, however, it was possible to obtain information on the true susceptibility of certain varieties of cereals and on various range and pasture grasses that usually escape severe injury in the field because of such conditions as late maturity or reduced humidity brought about by sparse stand.

In most of the more recent inoculation studies with *Cercosporella herpotrichoides* the inoculum was laid at the base of the culms about 60 days after the emergence of the plants and was then lightly covered with moist soil. This method was particularly necessary in handling grasses with small seeds, because otherwise the mass of inoculum was somewhat toxic to the germinating seeds. The addition of inoculum to the plant after it has become established more nearly approaches the manner of infection in the field in the Pacific Northwest and results in less stunting of the plant than when the inoculum is applied at seeding time.

Several attempts to use conidia of *Cercosporella herpotrichoides*, grown on corn meal, for inoculation purposes were not consistently successful on winter wheat in the greenhouse, and the practice was discontinued.

The probable reduction in yield under greenhouse conditions due to *Cercosporella herpotrichoides* was calculated by means of a graduated scale in which the approximate reduction in potential yield was considered as follows: Plants dead by disease, 100 percent loss; very severely injured, 80 percent loss; severely injured, 60 percent loss; moderately injured, 40 percent loss; slightly injured, 20 percent loss. This scale differs somewhat from that used in the field (*16*) because of the severe injury and stunting encountered under optimum greenhouse conditions. It must be emphasized that the injury under the greenhouse conditions was very severe and losses up to 80 percent of the potential yield were not uncommon; in a few cases the plants were nearly all killed.

RESULTS

The results of inoculation studies with *Cercosporella herpotrichoides* on a number of gramineous hosts during the period 1930–31 in the greenhouse at Corvallis, Oreg., are presented in table 3. The plants were sown and inoculated on two dates, October 7, 1930, and January 10, 1931, and were harvested on January 18, 1931, and May 4, 1931.

TABLE 3.—*Disease reaction of cereals and other grasses inoculated with pure cultures of Cercosporella herpotrichoides in the greenhouse at Corvallis, Oreg., 1930–31*

| Host (species and variety) | C. I. no. | Date sown | Culms showing indicated degree of injury | | | | | | | Estimated loss |
			Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*
Aegilops triuncialis		Oct. 7	14	58	29				101	22.9
Avena byzantina C. Koch (oats):										
Kanota	839	Jan. 10	23						23	.0
Red Rustproof		do	21	2	2	1			26	5.0
Avena fatua L. (wild oats)		do	34						34	.0
Avena sativa L. (oats):										
Winter Turf	1570	Oct. 7	123	2	1	1			127	1.1
Hordeum vulgare (barley):										
Alaska	4106	do	41	24	28	7	4	1	105	23.2
Do	4106	Jan 10	22	17	3		1	3	46	18.3
Tennessee Winter	257	do	23	12	6	3	2	4	50	24.4
Winter Club	592	Oct. 7	25	78	28	13	6		150	25.4
Secale cereale (rye):										
Oregon Winter		do	45						45	.0
Do		Jan. 10	21						21	.0
Triticum dicoccum (emmer):										
Black Winter	2337	Oct. 7	11	12	40	10	3	1	77	36.1
Do	2337	Jan. 10	11	11	3				25	13.6
Triticum durum (durum):										
Akrona	6881	Oct. 7	22	7	10	3	2	1	45	21.8
Kubanka	1440	Jan. 10	9	2	6	4		3	24	34.1
Triticum monococcum (einkorn)	2433	Oct. 7	19	37	55				111	26.5
Triticum spelta (spelt):										
Alstroum	1773	do	11	9	10	28	36	10	104	62.9
Do	1773	Jan. 10	6	5	8	18	2	1	40	44.0
Triticum aestivum (common wheat):										
Buffum No. 17	3330	Oct. 7	10	16	29	42	36	41	174	63.1
Federation	4734	do	3	9	11	8	7	12	50	61.2
Goldcoin (Fortyfold)	4156	do	2	3	24	25	13	4	71	55.8
Harvest Queen	5314	do	17	6	51	49	24	7	154	50.1
Hohenheimer	11458	do	2	17	37	29	27	6	118	53.6
Do	11459	do	2	0	22	30	15	10	79	61.7
Hosar	10067	do	2	5	23	35	27	8	100	60.8
Minhardi×Minturki	8215	do	2	1	18	15	39	7	82	66.5
Purplestraw	1915	do	1	5	24	28	21	40	119	77.2
Turkey	4429	do	40	14	12	20	32	14	132	44.9
Wilhelmina (Holland)	11389	do	0	5	32	28	17	11	93	59.4
Triticum compactum (club wheat):										
Hybrid 128	4512	do	15	6	21	21	21	9	93	51.6
Hybrid 143	4160	do	27	10	16	6	5	6	70	38.3
Jenkin	5177	do	7	2	9	8	6	0	32	42.5
Triticum aestivum×Secale cereale:										
Wheat-rye	8890	do	5	7	5	24	47	2	90	63.8

A similar experiment, which is summarized in table 4, was conducted in 1934–35. The grasses were seeded October 7 in the benches and inoculated after emergence on November 15 with pure-culture inoculum and in addition with cercosporella-infested soil taken from the surface soil of a heavily infested field at High Prairie, Wash. The plants were grown under 250-watt lights from 5 p. m. until 8 a. m. of each 24-hour period. The final records were taken February 19, 1935.

Other experiments on the resistance of species of Gramineae to *Cercosporella herpotrichoides*, which dealt especially with range and pasture grasses, were made at Corvallis and at the Arlington Farm in the greenhouse. The results of these experiments are given in tables 5, 6, and 7. The results presented in table 5 are from several species of Gramineae seeded October 1, 1931, in Newburg sandy loam soil in

rows spaced 4 inches apart in the greenhouse at Corvallis, Oreg.   The fungus was reisolated from diseased plants of each host.   All uninoculated checks remained healthy.

TABLE 4.—*Disease reaction of cereals and other grasses inoculated with pure cultures of Cercosporella herpotrichoides, and grown on soil infested with that organism, in the greenhouse at Corvallis, Oreg., 1934–35*

Host (species and variety)	C. I. no.	Culms showing indicated degree of injury							Estimated loss
		Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Aegilops triuncialis		4	4	10	52	5	8	83	42.4
Do					52	11	30	93	64.1
Avena byzantina (oats):									
Kanota	839	2	7	26	38	2		75	30.4
Do	839	2	2	6	38	12	2	62	39.0
Red Rustproof		12	28	16	7	2		65	16.0
Do			1	5	39	18	1	64	43.8
Avena fatua (wild oats)		10	18	17	15			60	18.6
Do		1	1	14	52	6		74	38.5
Avena sativa (oats):									
Winter Turf	1570	14	16	19	45	5		99	25.6
Do	1570	5	2	5	53	12		77	41.5
Hordeum vulgare (barley):									
Alaska	4106	3	6	12	33	6	13	73	60.3
Do	4106				48	8	15	71	57.4
Tennessee Winter	257		1	2	12	2		17	40.6
Do	257			1	10		9	20	66.0
Winter Club	592		5	15	52		1	73	34.7
Do	592				56	5	3	64	46.0
Winter (Wash. no. 2801)		10	12	36	23	2	3	86	25.8
Do		1	3	26	39	2	4	75	30.2
Secale cereale (rye):									
Oregon Winter		5		5	5			15	20.0
Do		4	1	4	4			13	19.2
Triticum dicoccum (emmer):									
Black Winter	2337	1	1	3	47	4	3	59	43.5
Do	2337		1	1	65			67	39.2
Triticum durum (durum):									
Akrona	6881	3	2	13	67	14	1	100	41.8
Kubanka	1440	6	2	9	31	5	2	55	37.1
Triticum monococcum (einkorn)	2433	5	6	32	41		4	88	31.1
Triticum spelta (spelt):									
Alstroum	1773	7	3	13	79	5	1	108	36.5
Do	1773	3	2	2	55	14	5	81	48.0
Bearded winter		1	1	2	47	25	13	89	58.7
Triticum aestivum (common wheat):									
Federation	4734			3	48	12	3	66	48.6
Goldcoin (Fortyfold)	4156				10	30	51	91	86.8
Hohenheimer	11458	1	3	5	43	26	42	120	67.7
Hosar	10067	7	6	4	55	13	8	93	44.9
Minhardi × Minturki	8215	2	5	9	56	25	84	181	71.1
Purplestraw	1915				30	10	40	80	75.0
Ridit	6703				26	33	29	88	74.7
Turkey	4429		1	3	25	31	64	124	88.3
Wilhelmina (Holland)	11389		1		29	16	28	74	70.9
Triticum compactum (club):									
Hybrid 128	4512	3	5	5	53	13	4	83	44.7
Do	4512			4	61	8	6	79	47.6
Do	4512			1	53	8	6	68	49.7
Do	4512		2	3	45	10	7	67	50.4
Do	4512				54	5	14	73	54.2
Do	4512				42	8	10	60	54.5
Do	4512	1			68	4	25	98	56.5
Do	4512		1	1	38	5	14	59	56.7
Do	4512				46	25	10	81	59.7
Do	4512	1	1	2	28	33	10	75	64.0
Do	4512		1		28	39	14	82	68.9
Hybrid 143	4160	9	5	8	41	3	1	67	32.6
Jenkin	5177		1	3	71	10		85	43.6

TABLE 5.—*Disease reaction of several species of Gramineae inoculated with pure cultures of Cercosporella herpotrichoides in the greenhouse at Corvallis, Oreg., 1931–32*

Host	Culms showing indicated degree of injury							Esti-mated loss
	Healthy	Slight	Mod-erate	Severe	Very severe	Dead	Total	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Agropyron repens (L.) Beauv	44	15		1			60	6.0
Agrostis palustris Huds	100		1	1	2		104	2.5
Bromus mollis L	40	4	2	1			47	5.5
B. rigidus Roth	55	22					77	5.7
B. secalinus	14	7	6	27	26	14	94	58.3
B. tectorum	25	19	3				47	10.6
Lolium multiflorum Lam. (im-ported)	65	25	15	16	2		123	18.7
L. multiflorum (western)	17	21	21	27	4		90	35.5
Triticum monococcum (einkorn)	4	57	7				68	20.9
T. aestivum (Kharkof)	1	3	4	5	5	15	33	73.3

A number of additional species of grasses were seeded in Newburg sandy loam soil in greenhouse benches at Corvallis, Oreg., on October 19, 1933. The rows were spaced 7 inches apart. Inoculum was added to the soil around the plants on December 18, and they were harvested March 14, 1934. Results from this test are given in table 6. Reisolations were positive from all hosts that showed more than 1 percent average loss. All uninoculated check plants remained healthy.

TABLE 6.—*Disease reaction of several species of Gramineae inoculated with pure cultures of Cercosporella herpotrichoides in soil in the greenhouse at Corvallis, Oreg., 1933–34*

Host	Reaction to inoculum from—			
	Triticum aestivum		*Bromus tectorum*	
	Infected culms	Esti-mated loss	Infected culms	Esti-mated loss
	Number	*Percent*	*Number*	*Percent*
Agropyron cristatum	55	12.1	124	10.0
A. intermedium (Host) Beauv	54	8.8		
A. smithii Rydb	106	12.5		
A. spicatum (Pursh) Scribn. and Smith	63	5.8	11	25.4
A. subsecundum (Link) Hitchc	34	7.9		
A. tenerum Vasey	63	6.2		
A. violaceum (Hornem.) Lange	78	18.7		
Agrostis alba L	199	0	212	0
Bromus rigidus	168	2.2		
B. secalinus	100	3.6		
B. tectorum	116	4.3	97	5.2
Cynosurus cristatus L	187	.5	156	0
Dactylis glomerata L	111	0	85	0
Festuca elatior			97	4.3
F. ovina L	94	0	92	3.0
F. rubra fallax Hack	143	0	172	
Lolium multiflorum			98	3.5
L. perenne L			103	4.4
Phalaris arundinacea L	82	.1	67	0
Phleum pratense L	177	0	105	0
Poa nemoralis L	145	.1	158	0
Poa secunda Presl			383	3.1
Poa trivialis L	235	0		
Triticum monococcum	87	17.1		
T. aestivum (Golden)	145	47.1		
T. aestivum (Huston)			56	52.5
T. aestivum (Hybrid 128)	96	51.6	129	56.6

A number of species of *Aegilops*, *Haynaldia*, and *Triticum* were planted in soil in the greenhouse at Arlington Farm on November 12, 1932, inoculated in the usual way with pure cultures of *Cercosporella herpotrichoides*, and harvested February 17, 1933.[5]   The infection data are given in table 7.

TABLE 7.—*Disease reaction of several species of Gramineae inoculated with pure cultures of Cercosporella herpotrichoides in the greenhouse at the Arlington Experiment Farm, Arlington, Va., 1932–33*

Host	Culms showing indicated degree of injury							Estimated loss
	Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Aegilops aucheri Boiss. var. *virgata*..	---	2	4	20	8	3	37	63.2
A. bicornis (Forsk.) Jaub. and Sp...	1	5	6	3	5	5	25	56.8
A. ovata L. var. *umbonata* Zhuk....	4	12	7	3	-------	-------	26	27.0
A. squarrosa L. var. *typica*...	22	11	3	-------	-------	-------	36	9.4
A. tauschii Coss.....	---	3	6	4	1	7	21	62.9
A. triaristata Willd....	6	10	3	-------	-------	-------	19	16.8
A. triuncialis...	12	20	11	8	2	-------	53	27.9
A. uniaristata Vis...	15	5	6	2	-------	1	29	16.0
A. variabilis Eig...	---	-------	---	---	1	7	8	97.5
A. ventricosa Tausch...	55	2	1	1	-------	-------	59	2.5
Haynaldia villosa (L.) Shur...	51	6	1	-------	-------	-------	58	2.7
Triticum dicoccum [1]...	-------	-------	-------	7	8	21	36	87.7
T. dicoccum var. Vernal...	-------	-------	-------	3	11	---	14	75.2
T. dicoccoides Koern...	-------	-------	-------	13	2	1	16	65.0
T. orientale Percival...	-------	-------	-------	4	2	-----	6	66.7
T. persicum Vav. var. *rubiginosum*..	-------	-------	-------	4	1	4	9	80.0
T. timopheevi Zhuk. var. *veliculosum*.	-------	-------	-------	6	2	7	15	81.3
T. aestivum var. Hybrid 128. ...	1	9	13	16	4	5	48	51.6
T. aestivum var. Chinese...	-------	-------	5	12	4	9	30	71.3
T. aestivum × *Secale cereale* [2]...	18	2	-------	-------	-------	-------	20	2.2

[1] Sando's awnless emmer.
[2] Meister's amphidiploid.

POSSIBLE ORIGIN OF CERCOSPORELLA FOOT ROT IN THE PACIFIC NORTHWEST

The first authentic collection of cercosporella foot rot in the United States was made on one of the prairies northeast of Hillyard near Spokane, Wash., in 1919 (7).   The disease was reported a short time later from Klickitat County, Wash. (8), and Union County, Oreg. (9), and since then from a number of localities (14, 16).[6]   Pioneer wheat growers are agreed that the disease was not widespread before 1910 and when present occurred on north slopes near the bottom of draws where moisture was relatively abundant but not excessive.   All evidence seems to indicate that the disease has been present for a long period on native grasses in the Columbia Basin and that the causal fungus has adapted itself to the more humid conditions of the wheat fields from a former struggling existence on native grasses where it had persisted even under semiarid conditions.   The fact that the disease is closely confined to certain isolated even though moderately extensive areas of a certain vegetation type in the Pacific Northwest strongly supports this view.

In the greenhouse all the species of *Agropyron* that were inoculated were susceptible to *Cercosporella herpotrichoides*, this grass ranking

[6] See footnote 3.

as one of the most susceptible of all the grasses tested. It is possible, therefore, that *A. spicatum* and *A. inerme* may be native hosts of this fungus.

DISCUSSION

RELATIVE SUSCEPTIBILITY OF CEREALS

The field and greenhouse studies show not only that certain varieties of wheat are highly susceptible to *Cercosporella herpotrichoides* but that those varieties that do escape disease in the field are often readily attacked by the fungus under conditions more favorable for the disease. For example, Wilhelmina, which is a late-maturing wheat with heavy straw, normally escapes serious injury in the field, but under optimum conditions for the fungus such as prevailed in the greenhouse at Corvallis it becomes seriously diseased. Following the mild, open winter of 1933–34 Wilhelmina and Red Russian, which seldom show serious injury in the field, were heavily diseased in the fields near Nezperce, Idaho. This tends to confirm the results of the greenhouse studies. In the greenhouse the same conditions held true for other late-maturing varieties of wheat, such as Hohenheimer and Hosar.

When sufficient inoculum is added to the soil such cereals as oats or einkorn and Oregon winter rye, which usually show little injury in the field, become heavily infected (table 4).

The value of the greenhouse studies lies in their disclosing the lack of true resistance in certain varieties in the field, information that is important in a breeding program.

RELATIVE SUSCEPTIBILITY OF OTHER GRASSES

As shown in tables 3 to 6, many species of grasses are susceptible to *Cercosporella herpotrichoides* when grown in crowded rows under optimum conditions for infection in the greenhouse. In general, however, the range and pasture grasses are distinctly more resistant than most varieties of wheat, and some wiry-leaved grasses, such as a number of species of *Festuca*, are highly resistant. Certain of the wheat relatives listed in table 6 also seem to show some resistance. While the number of culms that were tested in this experiment was very small, the conditions were so nearly ideal for maximum development of the disease that the results are considered as strongly suggestive. Some of these wheat relatives, such as *Aegilops ventricosa*, may have some value in breeding for resistance to cercosporella foot rot. This process would be very slow and uncertain, however, inasmuch as field experience with cereals bearing *Aegilops* parentage has been somewhat discouraging. Most of these hybrids that show resistance to cercosporella foot rot produce low yields, and those that produce fair yields assume the susceptible characteristics of the wheat parent.

On account of the need for widely spaced seeding in the Columbia Basin, in addition to the moderate resistance of the species, most of the adapted grasses other than cereals can be sown on cercosporella-infested ground with little danger of important losses from cercosporella foot rot.

SUMMARY

Cercosporella herpotrichoides Fron has been isolated from a few wild grass hosts growing in or at the edge of fields of winter wheat that were severely infested with the fungus.

The reaction to *Cercosporella herpotrichoides* of a number of cereals and other grasses in the field is presented in tabular form for each year for the period 1930–35. Because of semiarid conditions it is necessary to seed grasses sparsely. This assists aeration and precludes any great danger of loss from *C. herpotrichoides*, notwithstanding the moderate susceptibility of these grasses under greenhouse conditions optimum for the disease.

The results obtained in the greenhouse under optimum conditions for the disease were of special value in showing that certain late-maturing varieties of cereals that appeared resistant under field conditions in reality escaped the disease and do not contain genetic factors for resistance. The so-called resistance of these varieties under field conditions is considered to be due to escape through their lateness in maturing.

Various species of *Aegilops* and *Triticum* showed great differences in reaction to *Cercosporella herpotrichoides*, some being markedly resistant and some very susceptible. There is a possibility that resistant cereals may eventually be developed from hybrids between wheat and certain related wild grasses.

In the greenhouse under conditions optimum for the disease a number of genera and species of grasses were moderately susceptible to *Cercosporella herpotrichoides*. Species of *Agropyron* were found to be particularly susceptible.

Indications are that cercosporella foot rot has been present for a long period on grasses native to the prairies of the Columbia Basin.

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INTRASPECIFIC AND INTERSPECIFIC AVERSION IN DIPLODIA[1]

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INTRODUCTION

In the course of an extensive plating program involving the identification of fungi associated with the ear-rot diseases of dent corn, strains[3] of *Diplodia zeae* (Schw.) Lév.[4] were observed which in culture manifest intraspecific aversion, a phenomenon infrequently reported in fungi. It was observed that when adjacent colonies of *D. zeae* appeared in the culture plates one of two contrasting reactions occurred where the mycelia came together: The hyphae either intermingled freely, with a piling up or "drifting" of the mycelium at the line of juncture, or, more frequently, an antagonistic reaction took place in which growth at the tips ceased and was apparently followed by a dying back of the mycelium. This was followed by a darkening of the agar between the averting colonies, delimiting them cleanly from each other (fig. 1). The aversion phenomenon occurred upon all culture media tried, including potato-dextrose agar, oatmeal agar, and Coons', Richards', and Czapeks' media. Aside from the fundamental aspects of the problem, further studies on aversion in this economically important fungus seemed justified on the basis of practical considerations, especially in relation to pathogenicity studies and inoculation technique. The present paper includes the results obtained in studies involving (1) stability of strains of *D. zeae* for aversion reactions, (2) number and distribution of strains, and (3) inoculation experiments with single strains and mixtures of strains of *D. zeae* and *D. macrospora.*

OCCURRENCE OF INTRASPECIFIC AVERSION IN FUNGI

A study of the literature reveals a growing list of cases in which aversion has been observed within specific lines of fungi. The cases cited below are those which the writer believes bear analogy to the situation in *Diplodia* to the extent that the manifestation of the phenomenon appears to be dependent upon genetic differences in the cultures involved. Cayley (*3*)[5], in 1923, first directed particular attention to this phenomenon in her studies with *Diaporthe perniciosa.* In a later paper Cayley (*4*) cites, as additional cases, those described by Brunswik in *Coprinus fimetarius* and in *C. friesii;* by Vandendries in *C. micaceus, C. radians,* and *Panaeolus complanatus;* by Nakata in *Sclerotium rolfsii;* and by Mounce in *Fomes pinicola.* More recently

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cases of intraspecific aversion have been reported by Vandendries in *Pleurotus columbinus* (8) and *P. ostreatus* (9), by Vandendries and Brodie (10) in *Lenzites betulina*, and by Brodie (1) in *Corticium calceum*.

STABILITY OF AVERSION REACTIONS OF STRAINS OF DIPLODIA ZEAE

Twenty-one cultures of *Diplodia zeae*, including 12 strains, all isolated from rotted kernels of dent corn, were used in tests for studying the stability of the particular aversion reactions. Stability of reaction was maintained in every culture, (1) following mycelial propagation, (2) through successive pycnidial generations, and (3) following reisolations from inoculated corn plants. Two-percent potato-dextrose agar was used in all plating tests on stability.
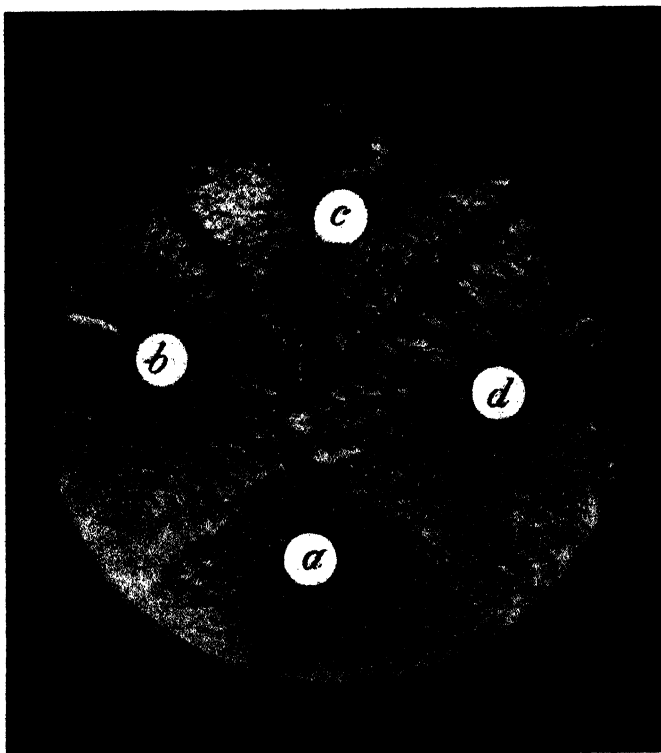
FIGURE 1.—Plate cultures showing aversion between strains of *Diplodia zeae*. Aversion is shown between colony *c* and colonies *b* and *d*. Colonies *a*, *b*, and *d* are of the same strain and show no aversion to one another. Colonies *a* and *c* would show aversion to each other if their mycelia came together. Note the intermingling or "drifting" of the mycelium between colonies *a* and *b* and between *a* and *d*.

The question first studied was whether two cultures of *Diplodia zeae* that show aversion to each other are of the same strain or of different strains. Plating mycelial inocula from any single culture of *D. zeae* at opposite sides in a Petri dish always resulted in a free intermingling of the hyphae, with a piling up or drifting of the mycelia where they met. In contrast, when mycelial inocula from two averting cultures were plated at opposite sides in a Petri dish, the characteristic

aversion reaction always occurred. It naturally followed that all
isolates of a given strain, wherever obtained reacted alike toward
other cultures. For example, if cultures A and B were found to be
alike, and if C and D also were alike, then, if further platings showed
A and C to be alike, it was invariably found that B and D likewise
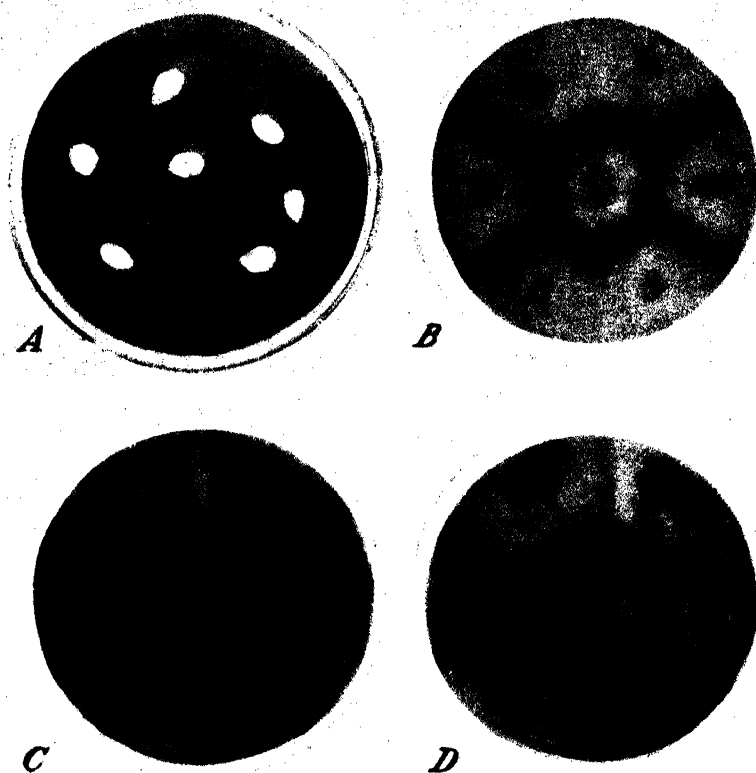
FIGURE 2.—Stability of aversion reactions in strains of *Diplodia zeae* as shown by plate cultures. *A*, Seven
original colonies of *Diplodia* growing from corn kernels. *B*, The same cultures following propagation by
means of mycelial transfers. *C*, Cultures again combined following three successive pycnidial generations.
Monospore cultures were used throughout. *D*, The same cultures again combined following reisolations
from inoculated ears of corn. The cultures in *C* were used for pycnospore production for the inoculations,
therefore four pycnidial generations actually were involved in these stability studies.

would intermingle, and that any one of the four would react in exactly
the same manner as any of the others when plated against any different
strain.

STABILITY FOLLOWING MYCELIAL PROPAGATIONS

The 21 cultures used for the studies on stability following mycelial
propagation were propagated in groups of 7 in each culture plate.
The three groups involved five, four, and three different strains,
respectively, which were so arranged on the plates that each group
presented its characteristic design as a result of the reactions between
the strains. Mycelial transfers to other plates, with the cultures
placed in the same relative positions, resulted in every case in the same
type of design as that in the original plate (fig. 2, *B*). This evidence

of stability was maintained in all cultures through each of three successive transfers.

STABILITY THROUGH SUCCESSIVE PYCNIDIAL GENERATIONS

After the cultures had been propagated three times by means of mycelial transfers, transfers were made to oatmeal agar to induce abundant pycnidial development. Three monosporic cultures were obtained from each of 3 randomly selected pycnidia from each of the 21 cultures. Plating the three cultures from a given pycnidium against one another resulted always in intermingling of the hyphae when the cultures met, indicating a uniformity in reaction for all spores from a given pycnidium. A similar lack of aversion resulted when representative colonies from the three pycnidia from a given culture were plated against one another, indicating that all the pycnidia from that culture were alike. Finally, plating the various cultures in their respective original positions resulted again in the designs originally observed in each plate group (fig. 2, *C*). This procedure was carried through three successive pycnidial generations with no changes in the behavior of any of the progenies, thereby demonstrating the stability of the aversion reactions of the strains through three pycnidial generations.

STABILITY FOLLOWING REISOLATION FROM INOCULATED CORN PLANTS

Cultures from the third pycnidial generation were used for field inoculations to study possible changes in reactions following passage through the host. The seven cultures illustrated in figure 2, *C*, were used in this experiment. A spore suspension from each of the cultures was injected with a hypodermic needle into the tips of five ears in each of three inbred lines of dent corn. Platings of the reisolated cultures against the original stock cultures proved that no changes had occurred in the reactions of any of the strains. When the isolates again were combined on plates in their original relative positions, the same design again was obtained (fig. 2, *D*), thus demonstrating the stability of the strains through the pathogenic phase of their life cycles.

NUMBER AND DISTRIBUTION OF STRAINS

Some information on the extent and distribution of strains of *Diplodia zeae* was obtained from experiments in which randomly selected cultures were paired on plates in all possible combinations. Twenty-five cultures of *D. zeae*, isolated from 25 ears of corn collected from 1 field near Bloomington, Ill., were used for a so-called local test. The results from the platings showed 1 strain to occur 3 times, 2 others to occur twice each, and 18 to occur but once, thus making a total of 21 strains in this random group of 25 cultures. For a regional test 25 cultures of *D. zeae* were obtained from as many widely separated points throughout the Corn Belt. In this experiment only two of the cultures proved to be of the same strain. Observational evidence from plating many hundreds of colonies of *D. zeae* from damaged kernels in terminal market samples of corn indicates further the existence of a multiplicity of strains. Aversion between adjacent colonies has usually been the rule, intermingling being relatively rare.

INOCULATIONS WITH MIXTURES OF STRAINS

Inoculation experiments involving mixtures of strains of *Diplodia zeae* suggested two problems of particular interest: (1) Would aversion function in the host tissues to such an extent that one strain would inhibit the further development of the others and thus singly overrun the host? (2) If this should prove to be true, would it be possible to use such mixtures for determining the relative pathogenicity of strains? In field experimental work, a mixture of several cultures frequently has been used on the assumption that this provided a safeguard against the use of an inferior culture.

EXPERIMENTS IN 1934

Three strains of *Diplodia zeae* isolated from rotted corn kernels from Maryland, Ohio, and Illinois were used in a mixed-strain experiment conducted at Madison, Wis., in 1934. In future references these strains are called nos. 150, 26, and 73, respectively. Two inbred lines of corn, namely, R4, which is resistant to ear infections of *D. zeae*, and Lan, which is susceptible, were inoculated by hypodermic injections of spore suspension into the tips of the ears while in the milk stage of development. Care was taken that the cultures were of the same age and that the inocula used were of approximately equal spore concentrations. Inoculations first were made with single cultures to test for pathogenic differences between the fungus strains, after which equal volumes of the remaining inocula were combined and used for the mixed-strain inoculations. The inoculated ears were harvested about 6 weeks later. It was apparent that the extent of rotting that had developed in the ears was no more severe when the mixture of strains was used than when the strains were used separately. Nor were any decided differences in pathogenicity evident between the three strains used. These relationships held in both the resistant and susceptible inbred lines.

The identity of the fungus strains recovered from the rotted ears was established by means of a series of platings that involved, finally, the pairings of representative cultures from each ear against the stock cultures of the strains used in the experiment. Five kernels, taken spirally from each of the rotted ears, were used as a basis for the determination of the strain or strains in the ear. The results from the identification of the reisolated cultures are given in table 1.

TABLE 1.—*Strains of Diplodia zeae recovered from ears of corn inoculated with single strains and with a mixture of these strains at Madison, Wis., 1934*

Strains used for inoculum	Inbred[1] lines inoculated	Ears inoculated	Inoculated ears from which indicated strains were recovered		
			No. 150	No. 73	No. 26
Single strains:		*Number*			
No. 150	{R4	15	15		
	{Lan	15	15		
No. 73	{R4	15		15	
	{Lan	15		15	
No. 26	{R4	15			15
	{Lan	15			15
Mixed strains:					
Nos. 150, 73, and 26	{R4	15			14
	{Lan	14		²1	14

[1] R4 is resistant and Lan is susceptible to diplodia ear rot.
[2] Tip kernels of 1 ear plated strain 73; the remainder of the ear plated strain 26.

It will be observed from table 1 that the strain recovered from each of the single-strain inoculations was the same as that used in the inoculum. This was to be expected in view of the stability of the aversion reactions of strains of *Diplodia zeae* as established in the earlier experiments. The data also show that in the mixed-strain inoculations, with one exception, strain no. 26 was the only one reisolated, and apparently it had completely inhibited the development of strains nos. 150 and 73, both in the resistant and in the susceptible inbred line of corn.

EXPERIMENTS IN 1935

In view of the consistency with which strain no. 26 inhibited the others in the mixed inoculations in 1934, it was decided to repeat the experiment with certain modifications the following season. As no differentials in reaction between inbred lines and fungus strains were obtained in 1934, it was decided to use but one inbred, Wis. R3, in 1935, and to concentrate on a somewhat more elaborate series of strain mixtures.

A strain of *Diplodia macrospora* was added to the three strains of *D. zeae* previously used for observations on interspecific aversion. In addition to the single-strain inoculations, the following mixtures were used: *D. zeae*, nos. 150 and 73; 150 and 26; 73 and 26; 150, 73, and 26; and *D. macrospora* and *D. zeae* nos. 150, 73, and 26. The methods for inoculation and for identification of cultures recovered were similar to those of the previous season. The results from the experiments are given in table 2.

TABLE 2.—*Strains of Diplodia zeae and D. macrospora recovered from ears of corn of inbred line Wis. R3 inoculated with single strains and various mixtures of these strains and species, at Madison, Wis., 1935*

Species and strains used as inoculum	Ears inoculated	Inoculated ears from which the indicated strains were recovered				
		D. macrospora	D. zeae no. 150	D. zeae no. 73	D. zeae no. 26	Mixture of strains
Single strains:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
D. macrospora	14	[1] 12	0	0	0	0
D. zeae no. 150	14	0	14	0	0	0
D. zeae no. 73	15	0	0	15	0	0
D. zeae no. 26	11	0	0	0	11	0
Mixed strains:						
D. zeae nos. 150 and 73	15	0	0	15	0	0
D. zeae nos. 150 and 26	13	0	0	0	13	0
D. zeae nos. 73 and 26	15	0	0	1	14	0
D. zeae nos. 150, 73, and 26	36	0	0	0	28	[2] 8
D. macrospora and D. zeae nos. 150, 73, and 26	33	0	2	1	24	[3] 6

[1] Of 14 ears inoculated 12 developed ear rot.
[2] Strain no. 26 predominated in 6 of the 8 ears from which more than 1 strain was isolated. Strain no. 73 predominated in 2 of the 8 ears. Strain no. 150 was isolated from tip kernels in only 1 ear.
[3] *D. zeae* no. 73 predominated in 4 of the 6 ears from which more than 1 strain was isolated. *D. zeae* no. 150 predominated in 2 of the 6 ears. *D. zeae* no. 26 was isolated from all 6 of the ears. *D. zeae* nos. 73, 150, and 26 were all isolated from 1 ear. *D. macrospora* was absent in all ears.

Except for *Diplodia macrospora*, which appeared decidedly less pathogenic under the conditions of this experiment, no differences in extent of ear rot were evident in any of the inoculations, either in the comparisons between strains or between the various mixtures of

strains. The data in the table show that the cultures recovered from the single-strain inoculations were the same as those used in the inoculum, again demonstrating stability following passage through the host.

Of the three strains of *Diplodia zeae*, no. 73 alone was recovered from the 15 ears inoculated with a mixture of nos. 73 and 150. Apparently it had completely inhibited the development of the latter. In a similar manner strain no. 26 completely inhibited the development of strain no. 150 in all of the 13 ears inoculated with a mixture of the two strains. Strain no. 26 inhibited strain no. 73 in 14 out of 15 ears inoculated with this mixture and was itself inhibited by strain no. 73 in the 1 remaining ear. Thus a definite sequence in inhibitory effects occurred among these three strains, no. 73 inhibiting no. 150, and no. 26 inhibiting both no. 73 and no. 150.

Strain no. 26 in most instances maintained its inhibitory powers in the mixture involving all three strains. It alone was recovered from 28 of the 36 ears inoculated with this mixture. In addition to no. 26, other strains also were recovered from each of the remaining eight ears of this group. Strain no. 26 predominated on six of these ears (it alone was plated in the region between the middle and the butt of the ear); strain no. 73 predominated on two of the ears; and strain no. 150 was recovered from tip kernels of only one ear.

The results from the mixture involving the three strains of *Diplodia zeae* and one strain of *D. macrospora* again showed *D. zeae* no. 26 to be the dominating one. It alone was recovered from 24 of the 33 ears in this group. *D. zeae* no. 150 was the only one recovered from two ears, and *D. zeae* no. 73 was the only one recovered from one ear. *D. zeae* no. 26 also was recovered from each of the remaining six ears that yielded more than one strain of the fungus. *D. zeae* no. 73 predominated on four of these ears, and *D. zeae* no. 150 predominated on the remaining two ears. In no case was *D. macrospora* recovered.

These experiments demonstrated that *Diplodia zeae* no. 26 was able to manifest its inhibitory powers, in the various mixtures of strains used, in two successive seasons and in three inbred lines of corn.

DISCUSSION

The occurrence of intraspecific aversion in *Diplodia* represents an exception to the general rule as stated by Porter (7), i. e., that antagonism between fungi becomes more marked as the phylogenetic relationships are widened. It is beyond the scope of the present paper to discuss the hypotheses that have been advanced to explain aversion, but it is desirable to point out an important and basic difference between aversion in *Diplodia* and a phenomenon sometimes observed within specific lines in other fungi. Accepting Brown's definition (*2, p. 106*) of staling as the effect of "those metabolic products of the organism responsible for slowing down or stopping its growth", in the opinion of the author it becomes apparent that aversion in *Diplodia* is not due to staling products. Aversion in *Diplodia* results from the interaction between two physiologically different strains. In some other genera an entirely different situation exists, which might be confused with intraspecific aversion. In *Chaetomium* sp., for example, a very distinct inhibition of growth occurs when two colonies approach each other in culture. However, an identical reaction is observed

when a given culture is plated against itself. This, it seems, is either a nutritional response, or, more probably, a reaction closely allied to Brown's conception of staling. In either case it does not establish the existence of distinct physiological differences such as appear to exist in the strains of *Diplodia*.

In the literature dealing with intraspecific aversion the aspect of the problem studied most intensively concerns the relationship between the phenomenon and sex. Among studies on the inheritance of the phenomenon and its complex sexual relationships in Ascomycetes and in Hymenomycetes are papers by Cayley (*3, 4*), Vandendries (*8, 9*), and Vandendries and Brodie (*10*).

Little of the previous work on aversion is directly analogous with the studies reported herein with *Diplodia*. The fact that like meets like with intermingling of the mycelia and that aversion results when unlike strains meet has been reported by Cayley (*3*) in a homothallic form of *Diaporthe perniciosa*, by Nakata (*6*) in *Sclerotium rolfsii*, and by Mounce (*5*) in *Fomes pinicola*. A case in which unlike strains sometimes intermingle is described by Cayley (*4*), and this she believes to be a heterothallic form of *Diaporthe*. In this instance mycelia which meet can segregate into averting and nonaverting strains in later perithecial generations.

The uniformity in reaction of all monosporic mycelia from individual pycnidia in *Diplodia zeae* is in agreement with Cayley's (*3*) results with *Diaporthe*.

The multiplicity of strains found in *Diplodia zeae* agrees generally with studies on this aspect by Cayley (*3*), Mounce (*5*), and Nakata (*6*), with *Diaporthe perniciosa*, *Fomes pinicola*, and *Sclerotium rolfsii*, respectively.

No previous accounts of mixed-strain inoculations are known to the writer. Cayley (*4*) raised the question whether the aversion reaction might possibly be inhibited in host tissues owing to absorption of the toxins secreted by the fungus. A case of aversion between colonies of *Corticium calceum* on a piece of pine wood was recently described by Brodie (*1*). He believed this to be the first reported case of the occurrence of the phenomenon in nature.

The results from the mixed-strain inoculations with *Diplodia* indicate that the aversion reaction functions within the host. The consistency with which a strain eventually gets the upper hand and rots the major portion of the ear can hardly be explained on any other basis. The writer does not recall having plated more than one strain of *D. zeae* from naturally infected ears, except possibly where separate infections had occurred, one at the tip and another at the butt end of the ear.

No conclusions could be drawn from the mixed-strain experiments regarding the relationship between ability to inhibit and degree of pathogenicity. Despite the definite sequence in inhibitory powers manifested among the strains in the mixed inoculations, no appreciable differences in extent of ear rot were apparent when the strains were used singly in inoculations. It is quite conceivable, however, that an improved technique for comparing relative pathogenicity might reveal differences not apparent in these experiments. On the other hand, it is possible that physiological studies on the fungus strains might show differences in rates of spore germination and subsequent growth which might be correlated with their so-called inhibitory powers.

The results from the inoculations with mixtures of strains indicate that, for practical purposes, the use of mixed cultures accomplishes little beyond the diluting of the dominating strain used. The method does provide a technique for the selection of a very dominating strain for inoculations in experimental work.

The many strains of *Diplodia zeae*, each of which apparently remains constant or stable for its aversion character, raises a question as to the origin of all these strains. If the capacity of strains to inhibit others is an attribute of *Diplodia* strains generally, should not the strains in a given region have become limited through natural selection to a relatively small number? This situation seemingly does not exist. The evidence reported herein indicates that the differences among the strains are genetic and suggest the possible existence in this species of a sexual stage not yet described.

SUMMARY

The phenomenon of intraspecific aversion, infrequently reported in fungi, has been found in *Diplodia zeae* (Schw.) Lev. and *D. macrospora* Earle, and interspecific aversion has been found between these two species.

The stability of the particular aversion reactions of the different strains of *Diplodia zeae* was maintained, after successive mycelial propagations, through three successive pycnidial generations in culture and through inoculation into ears of corn and subsequent reisolation.

The number of strains of *Diplodia zeae* apparently is very large. Twenty-one different strains were obtained from 25 cultures isolated from as many ears of corn collected in one field near Bloomington, Ill. Among 25 isolates from widely separated points throughout the Corn Belt, 24 strains were obtained.

Field experiments involving inoculations with single strains and with mixtures of strains were conducted at Madison, Wis., in 1934 and 1935. Inoculations with various combinations of three strains of *Diplodia zeae* and a strain of *D. macrospora* were made in three inbred lines of corn. Identification of the strains recovered from the subsequently rotted ears proved that inhibitory effects had occurred so that usually only one strain of the fungus could be reisolated from an ear. It was also found that a definite sequence existed in the inhibitory powers of strains upon one another. So far as tried, *D. zeae* predominated over *D. macrospora*.

No conclusions could be drawn regarding the relationship between the capacity of strains for inhibiting the development of others and their degrees of pathogenicity.

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THE EFFECT OF STORAGE UPON THE VITAMIN A CONTENT OF ALFALFA HAY [1]

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INTRODUCTION

In a previous paper from this station [2] the vitamin A content of alfalfa was shown to be affected by exposure of the leaves to sunshine during the curing process. At the time of that investigation it was observed that the vitamin A value of the alfalfa leaves had to be determined as soon as the hay reached the laboratory if consistent results were to be obtained. When the vitamin tests were repeated on the same samples of hay later in the year, the vitamin A values were lower. This suggested the advisability of studying the effect of storage upon the vitamin A content of alfalfa.

The studies of Hauge and Aitkenhead [3] indicated that enzymes are responsible for the destruction of vitamin A which occurs during the field-curing process, and it seemed logical to suppose that this destructive action might continue, though perhaps more slowly, in alfalfa stored under commercial conditions after baling. In 1930 Quinn, Hartley, and Derow [4] reported that spinach when finely ground and kept in a mason jar in the laboratory lost approximately 70 percent of its vitamin A during a 12- to 15-month storage period.

Guilbert [5] has recently made a study of the factors that cause variations in the carotene content of alfalfa hay and meals. On the assumption that variations in the carotene content are a criterion of variations in the vitamin content, he interprets his findings in terms of the effect of these factors upon the vitamin A value of alfalfa. Guilbert finds that temperature is the major factor causing a loss of carotene in alfalfa during storage and that the rate of loss is roughly doubled for each 10° C. rise in temperature.

This paper briefly reports the effect of storage of alfalfa hay in Arizona upon its vitamin A content as determined by biological assay.

EXPERIMENTAL MATERIALS AND METHODS

The alfalfa used was of the Hairy Peruvian variety produced at the Salt River Valley Experiment Farm, Mesa, Ariz. The hay was cut with a mower and left in the windrows until wilted. It was then raked, shocked in small cocks, cured, and baled. Immediately after baling, it was stored in a hay shed on the farm. At intervals of 3

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months, one bale of alfalfa from the same cutting and curing was sent
to the nutrition laboratory of the University of Arizona at Tucson.
In the laboratory the leaves of a representative sample of the bale
were carefully separated by hand from the stems and petioles so as
to obtain a uniform sample. This material was kept in covered glass
jars during the conduct of the test. The vitamin A measurement was
begun at once with animals that had been prepared in advance for
the test.

The first study was made on alfalfa baled August 9, 1932, and a
second was made the following year with the 1933 crop of alfalfa baled
August 11.

In the biological assay of the vitamin A content of the alfalfa, the
standard method of Sherman and Munsell, as modified somewhat by
Sherman and Burtis,[6] was used. The method consisted essentially in
feeding graded portions of alfalfa as the sole source of vitamin A to
rats which had been maintained upon a vitamin A-deficient ration
until their body stores of the vitamin had become exhausted. The
growth response to the alfalfa supplement given daily (6 days) during
an 8-week experimental period was used as the criterion of the amount
of vitamin A in the alfalfa fed. Representative animals from each
litter were kept as controls and other details of the test were conducted
in the usual manner.

EXPERIMENTAL RESULTS

The growth responses of the rats to the feeding of weighed amounts
of alfalfa varying from 0.005 to 0.050 gms are presented in summarized
form in table 1. It may be noted that the results for the crops of
1932 and 1933 are quite similar.

When the vitamin A test was begun in August upon the freshly
baled alfalfa, a high vitamin A potency was observed. Five milligrams
of the alfalfa leaves were found to induce a gain of 41 g during the 8-week
test. This is considerably greater than the unit rate of gain as defined
by Sherman of 25 g in 8 weeks. When the test was repeated in No-
vember on another bale of hay which had been stored as previously
described for 3 months, the growth response to the same levels of
alfalfa supplement was strikingly different. In November twice as
much alfalfa was required to induce approximately the same rate of
gain.

The vitamin A content determined in February of the alfalfa which
had been stored on the farm since August (a 6-month period) was
far lower than that of the freshly baled hay in August, but it was of
practically the same order as that obtained in November. The test
made at this time showed a gain of approximately 40+ g resulting
from the daily supplement of 10 mg of alfalfa for 8 weeks, or an
approximate unit rate of gain induced by 7.5 mg of this alfalfa.
Thus, no further destruction of vitamin A during the storage of alfalfa
for the 3 months, November to February, was apparent.

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TABLE 1.—*Summarized results of feeding alfalfa stored for different lengths of time to albino rats as the sole source of vitamin A*

Length of storage period (months)	1932 crop				1933 crop			
	Amount of alfalfa leaves fed daily	Rats used in test	Average gain or loss in weight	Survival in test period	Amount of alfalfa leaves fed daily	Rats used in test	Average gain or loss in weight	Survival in test period
	Grams	*Number*	*Grams*	*Days*	*Grams*	*Number*	*Grams*	*Days*
0	0	4	----	29	0	4	----	30
	.005	6	+41	----	.005	8	+45	----
	.0075	6	+63	----	.0075	9	+57	----
	.01	6	+71	----	.01	9	+68	----
3	.0	6	----	37	.0	8	----	25
	.005	10	+13	----	.005	11	----	53
	.0075	11	+26	----	.0075	9	+19	----
	.01	7	+38	----	.01	8	+40	----
6	.0	6	----	27	.0	8	----	23
	.005	7	----	45	.005	8	----	57
	.0075	5	+23	----	.0075	8	+29	----
	.01	5	+42	----	.01	8	+45	----
9	.0	8	----	27	.0	8	----	24
	.005	5	----	30	.005	8	----	37
	.0075	2	----	36	.0075	10	----	42
	.01	8	+8	----	.01	8	----	50
12	.0	8	----	32	.0	8	----	30
	.005	2	----	21	.005	6	----	36
	.0075	2	----	38	.0075	6	----	47
	.01	4	+15	----	.01	8	+21	----
	.02	6	+41	----	.02	8	+45	----
	.03	4	+58	----	.03	6	+65	----
	.05	10	+77	----	.05	4	+83	----

When the measurement was repeated 3 months later in May on alfalfa which had been stored for 9 months, the results showed an even greater loss in vitamin A potency. Unfortunately, in this case the highest level at which the alfalfa was fed, 10 mg daily, was not sufficient to induce unit rate of gain. Most of the animals did not survive the test period, and on autopsy all showed a condition typical of vitamin A deficiency. Storage of the alfalfa until August, a period of 12 months, resulted in a product which was very inferior to the freshly baled hay. It required the daily feeding of 20 mg of this hay to promote a gain of approximately 40 g in the test animals in the 8-week period, a rate of gain which resulted from the daily feeding of only 5 mg of the hay which was tested before storage. Thus it appears that hay stored in Arizona during the 3 months, August, September, and October, undergoes marked deterioration, the destruction of its vitamin A content amounting to 50 percent. Further storage during November, December, and January resulted in no further measurable destruction. The alfalfa tested after 6 months' storage was also found to contain about 50 percent less vitamin A than the original freshly baled alfalfa. When, however, the hay was stored for an even longer time, there was a further loss in vitamin A. From February to August there was another loss of 50 percent of the vitamin A, which was found in the alfalfa stored for 6 months, or until February. This meant that by the following August the alfalfa leaves contained but 25 percent as much vitamin A as the original material baled 12 months previously.

In general, these findings agree with the work of Guilbert, who found that the rate of carotene destruction in alfalfa varied with the season of the year. He reported a decrease of 9 percent in carotene content in chopped sun-cured alfalfa hay after storage in a dark loft in Davis, Calif., from November to April, and a further decrease of 30 percent in hay stored from April to August. His belief that the temperature of the environment is a major factor in the rate of carotene or vitamin A destruction offers a satisfactory explanation of the findings reported herein relative to the rate of destruction of vitamin A under storage conditions in Arizona. During the first 3-month storage period in 1932 the mean maximum temperature ranged from 101° to 83° F. as compared with a mean maximum temperature range of 77° to 58° in the following 3 months. The temperature in Mesa rises sharply in the spring, and May, June, and July are hot months, with a mean maximum ranging from 84° to 103°. The rate of destruction of vitamin A is thus seen to be rapid in hot weather, and slow or at a standstill during the winter months of storage.

CONCLUSIONS

Leaves of baled alfalfa stored from August to November in a hay barn in Mesa, Ariz., contained 50 percent less vitamin A than the leaves of freshly baled alfalfa, as determined by biological assay. No further destruction of vitamin A occurred during subsequent storage in the cold months of November, December, and January. With rising temperature in the spring, the destruction of vitamin A was again stimulated. After 12 months in storage the alfalfa contained only 25 percent as much vitamin A as had been found in the fresh alfalfa.

PHYTOPHTHORA ROOT ROT OF CAULIFLOWER [1]

By C. M. TOMPKINS, *assistant plant pathologist, California Agricultural Experiment Station;* C. M. TUCKER, *plant pathologist, Missouri Agricultural Experiment Station;* and M. W. GARDNER, *plant pathologist, California Agricultural Experiment Station* [2]

INTRODUCTION

A destructive root rot of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) occurs during the winter in certain coastal regions of California (*10*),[3] mainly in low or poorly drained spots in heavy soils that are subject to waterlogging during irrigation or rainy periods. Considerable losses as a result of this disease have been observed in all varieties in the San Francisco Bay section. The disease has also been found near Colma, Half Moon Bay, and Salinas. Other naturally infected hosts are cabbage (*B. oleracea* L. var. *capitata* L.), Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), and hybrid cineraria (*Senecio cruentus* DC.) grown in a lath house at Salinas. J. B. Kendrick isolated the fungus from diseased stock or gilliflower (*Mathiola incana* R. Br. var. *annua* Voss) collected in Solano County in January 1934.

The disease is caused by *Phytophthora megasperma* Drechsler.

SYMPTOMS

The disease is characterized by a reddish discoloration of the older leaves followed by a sudden wilting of all the older leaves, which fall prostrate to the ground (fig. 1), leaving the head or curd exposed. The curd is not noticeably discolored but becomes tough and rubbery and is unmarketable. Plants of all ages are susceptible.

The lower end of the taproot, often along with the underground part of the stem, is badly rotted (fig. 2), and infected plants may be pulled from the soil with little effort. The cortex of the taproot and lateral roots is softened and water-soaked (fig. 2, *B*), usually sloughs off, and remains in the soil when the plant is pulled. The woody cylinder or stele is discolored and often frayed at the lower end (fig. 2, *A*). About the upper edge of the diseased part of the root there may be callus formation in the cortex (fig. 2, *A*), and numerous adventitious roots are sometimes produced (fig. 2, *C*). The upper margin of the diseased tissues of the cortex, stele, and pith is marked by a blackened zone. The infected pith is often collapsed, with resultant cavities.

In earlier stages of infection, lateral cankers are found in the cortex of the root, but in plants that show leaf symptoms the entire root and part of the underground stem are usually involved. Externally, the

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diseased tissues may be Isabella color, sepia, or black; internally, ivory yellow to sepia of Ridgway (9).

Typically, the reddish discoloration of the older leaves consists of a broad, marginal band with interveinal extensions toward the midrib, the upper surface being Pompeian red to neutral red, the lower surface Vernonia purple.

THE CAUSAL FUNGUS, PHYTOPHTHORA MEGASPERMA

The fungus is readily isolated by planting tissue fragments taken from the margin of the lesion in malt-extract agar (7). The same fungus was obtained from diseased cauliflower plants collected in

FIGURE 1.—Phytophthora root rot of cauliflower in a low area in the field, February 14, 1934. The wilting is preceded by a reddish discoloration of the older leaves.

various localities and from cabbage, Brussels sprouts, stocks, and cineraria hybrids. It is found only in the root system and underground portions of the stem.

Freehand cross and longitudinal sections of naturally infected roots were stained with fast green and Magdala red. The fungus is intercellular. Drechsler (4) observed the mycelium of *P. megasperma* to be intercellular, and Dowson (3) found an abundant intercellular mycelium in decaying carrots.

The fungus grows rapidly on the ordinary culture media. On oatmeal agar it produces short, tufted aerial growth which gives the surface a somewhat mealy appearance. Sexual spores develop early and very abundantly on this medium. Transfers of isolates of *Phytophthora megasperma* from hollyhock, the type culture of Drechsler (4), stock, cabbage, cauliflower, cineraria, and of a culture (host not specified) which was sent to the Centraalbureau voor Schimmelcultures,

Baarn, Netherlands, by Ashby, behave similarly in cultural characters and spore production. Oogonia and oospores from oatmeal-agar cultures vary somewhat in size, as shown in table 1.

FIGURE 2.—Phytophthora root rot of cauliflower: *A*, Shredding of woody cylinder and callus at lower edge of healthy cortex; *B*, dark discoloration of rotted cortex; *C*, formation of adventitious roots above rotted portion of taproot.

TABLE 1.—*Diameter of oogonia and oospores of Phytophthora megasperma from various sources*

Source	Oogonial diameters		Oospore diameters	
	Limits	Average	Limits	Average
Cauliflower:	*Microns*	*Microns*	*Microns*	*Microns*
1	32–43.2	38.2	29.2–36.4	33.4
2	32–46.0	38.4	26 –40	33.2
Stock:				
1	30–48	42.8	26 –45.6	39.6
2	32–50	42.0	26 –44	37.6
Cabbage	32–43.6	38.0	28 –40	33.2
Hollyhock	36–52	42.6	32.8–45.2	37.2
Ashby culture	32–47.2	41.6	28 –41.6	36.0
Cineraria	31–41.7	35.9	29.7–38	33.1

The size of the oogonia and oospores is affected by temperature, smaller spores developing at temperatures near the maximum, and by the culture medium, those produced on moist corn meal being smaller than those produced on oatmeal agar. Oogonia and oospores produced in liquid media are generally slightly smaller than those produced on solid media. The size of the oogonia and oospores is, therefore, subject to variation. However, all observed are sufficiently larger than those of *Phytophthora cactorum* to permit ready separation of cultures of the two species. The oogonia of *P. megasperma* are thin-walled, usually spherical or subspherical, with a short, rather slender stalk (pl. 1), while those of *P. cactorum* are inflated in the region enclosing the oospore and taper to form a broad funnel-shaped stalk. The latter type often appears in cultures of *P. megasperma* but is not typical of the species. The oogonia of *P. syringae* and *P. hibernalis* resemble those of *P. cactorum*. The oospores of *P. megasperma* occur singly in the oogonia, almost filling them (pl. 1, *C–G*); the oospore wall is smooth, thick, lemon yellow to straw color. Germination was not observed.

The antheridia of *Phytophthora megasperma* are preponderantly paragynous in type (pl. 1, *B–G*), yet variable proportions of them are amphigynous (pl. 1, *H*). The latter type is produced more frequently in liquid than on solid media, and in many cases the oogonia and oospores are smaller than those with paragynous antheridia. In all cultures examined the paragynous greatly outnumbered the amphigynous antheridia. None of the latter was found in oatmeal-agar cultures of some of the isolates. The antheridia may be borne on different or the same hyphae on which the oogonia develop; sometimes the antheridia develop on a short branch originating on the same hypha and only a few microns from the point of attachment of the stalk of the oogonium.

Sporangia do not develop on the solid media used, and but very scantily in liquids. A few appeared on hyphae grown 4 days in pea broth and transferred to sterile distilled water, as suggested by Rands (*8*) in 1922 and more recently by Leonian (*6*). They were obpyriform with a broad rounded base, nonpapillate, but with a thin refringent layer at the apical end, resembling those described by Drechsler (*4*). They measured 41.6 to 56 by 28 to 40 microns, averaging 49.4 by 33.6 microns. Zoospore development and discharge were observed. Attempts to induce sporangium development by flooding cultures on Difco and unfiltered corn-meal agar and lima-bean agar with sterile distilled water and by transferring hyphae from oatmeal-agar cultures to Petri's solution and a nonsterile soil suspension were not successful.

The temperature relations of the cauliflower fungus are similar to those of the other isolates of the species, as indicated by the results of plate tests in which Difco corn-meal agar was used.

Of the other species with paragynous antheridia *Phytophthora hibernalis* and *P. syringae* cannot tolerate the high temperatures at which *P. megasperma* grows well. The temperature relations of *P. cactorum* are similar to those of *P. megasperma*.

The species appears to be quite distinct, and is one of the most easily recognized because of its large sexual spores which are produced early and profusely. It was not included in the key for the identifica-

PHYTOPHTHORA MEGASPERMA DRESCHLER. ✕465.

A, Young oogonium without antheridium. *B*, Unfertilized oogonium and antheridium. *C*, Fertilized oogonium with developing oospore. *D-G*, Oogonium with oospores in various stages of development, as indicated by the thickening of the walls of the oospores, the antheridia in *B-G* being paragynous. *H*, Oogonium with amphigynous antheridium.

tion of species published by Tucker (*11, p. 190*) in 1931, which may now be emended as follows:

B. Widely spreading growth on malt-extract agar and ordinary agar media after 6 days at 20° C.
   1. Oogonia developing promptly (within 2 weeks) and abundantly on oatmeal agar or steamed corn meal.
     Antheridia predominantly paragynous.
       a. Growth on corn-meal agar after 4 days at 28° C.
         (1) Average diameter of oospores (oatmeal-agar cultures) exceeding 30 microns.   *P. megasperma.*

         (2) Average diameter of oospores less than 30 microns.
                                             P. cactorum.
       b. No growth on corn-meal agar after 4 days at 28° C.   *P. syringae.*

TABLE 2.—*Diameter of mycelial growth of Phytophthora megasperma from various sources after exposure for 96 hours at different temperatures in plate tests*

Source	Diameter of mycelial growth after 96 hours at		
	20° C.	25° C.	30° C.[1]
	Millimeters	*Millimeters*	*Millimeters*
Cauliflower:			
1	25	25	18
2		38	23
Stock:			
1	30	38	33
2		40	21
Cabbage		33	20
Hollyhock		43	8
Ashby culture		41	22
Cineraria		44	28

[1] No growth at 35° C.

Since the isolation and description of *Phytophthora megasperma* by Drechsler (*4*) in 1931 as the cause of a crown rot of the hollyhock in the District of Columbia, the species has been found widely distributed and attacking hosts not closely related. Cairns and Muskett (*1, 2*) in 1933 reported the isolation of the species from potato tubers affected by a pink rot in Northern Ireland, and its identification by Ashby. Fawcett (*5*) in 1933 reported the isolation of the same species, also identified by Ashby, from citrus roots in California. Dowson (*3*) obtained the fungus in Tasmania, from the roots of carrots growing in a reclaimed bog during an abnormally wet season, under conditions resembling those prevailing in fields where the species causes a root rot of cauliflower in California. It is worthy of note that Cairns and Muskett (*2*) emphasize the importance of soil drainage "sufficiently good to prevent the land from becoming unduly wet in times of heavy rainfall" as a means of preventing pink rot of potatoes caused, at least occasionally, by *P. megasperma.*

INOCULATIONS IN WATERLOGGED SOIL

Attempts to cause infection of potted cauliflower plants in the greenhouse, by adding inoculum to the soil and watering the plants heavily, were unsuccessful, even when the roots were wounded by needle pricks. Typical infection occurred on unwounded roots, how-

ever, when the pots were set in about 4 inches of water in buckets and incubated outdoors in tests made during February to May. The incubation period was about 3 weeks.

Inoculum was prepared by growing the fungus isolated from cauliflower on sterilized, moistened, cracked wheat and adding this to the soil in 6-inch pots, each containing a young cauliflower plant growing in autoclaved soil. On January 7, 12 potted plants of the February variety of cauliflower were slightly wounded by pricking the taproot with a sterilized needle. After the inoculum had been added to the soil, the pots were placed in buckets out of doors. The temperature varied from 14° to 20° C. On February 10, 10 of the inoculated plants showed typical wilting and reddening of the leaves, while the 6 noninoculated controls remained healthy. The fungus was reisolated from each of the 10 infected plants.

On March 8, 1935, 15 potted cauliflower plants with six leaves were inoculated without wounding the roots and placed in buckets of water along with five noninoculated controls. On March 26, all of the inoculated plants were infected, and the fungus was reisolated from 12 plants. The controls remained healthy in spite of the waterlogged condition of the soil. Another set of 15 plants was similarly inoculated on March 15, and on April 9 all were infected. The cortical tissues were completely softened and were readily sloughed off from the central cylinder. The fungus was reisolated from these plants. The five noninoculated controls remained healthy. Similar results were obtained in a set of 20 plants inoculated with the reisolated fungus on May 8. On May 24, 19 were infected and the fungus was reisolated from 18 roots. The five noninoculated controls were healthy.

EXPERIMENTAL HOST RANGE

By the use of the inoculation method that provided waterlogged soil, the fungus isolated from cauliflower proved to be pathogenic to stock (*Mathiola incana* var. *annua*) and wallflower (*Cheiranthus cheiri* L.).

On March 8, 1935, 15 potted stock plants in the six-leaf stage were inoculated without wounding the roots and placed in buckets of water along with 5 control pots. On April 19, 13 of the inoculated plants were infected, and the fungus was reisolated from each. The five controls remained healthy. A repetition of the test was made on May 10 with a culture reisolated from stock, and by June 2, 7 of the 10 inoculated stock plants were infected, and the fungus was reisolated. The four controls remained healthy.

On April 6, 20 potted wallflower plants were inoculated and placed in buckets of water along with 5 noninoculated control pots. On April 27 all of the inoculated plants were infected, and the fungus was reisolated from 16. The controls remained healthy. On June 7, 20 wallflower plants were inoculated with a culture reisolated from infected wallflower plants and on July 8, 12 were infected. The fungus was recovered in culture. The noninoculated controls remained healthy.

Ripe fruits of tomato *Lycopersicum esculentum* Mill. var. *commune* Bailey) were successfully infected. Inoculations were made by placing a small square of inoculum from an agar culture of the fungus

on the unbroken surface and keeping this moist with absorbent cotton under an inverted preparation dish. On January 15, 1936, four fruits were inoculated with a 6-day-old agar plate culture. On January 24, water-soaked lesions had formed measuring 5.0 by 7.0, 6.0 by 4.0, 5.0 by 4.0, and 5.0 by 5.0 cm. The two fruits used as controls remained healthy. The fungus was reisolated from all infected fruits. With the reisolated fungus, four tomato fruits were inoculated on January 30. Two fruits were infected on February 2 and the fungus was reisolated.

On March 5, 1936, eight cineraria plants in the six-leaf stage were inoculated without wounding the roots, and six noninoculated plants were used as controls. On March 22 five of the inoculated plants were infected and the fungus was reisolated from each, while the controls remained healthy.

Attempts to infect unwounded fruits of bell or sweet pepper (*Capsicum frutescens* L. var. *grossum* (Bailey)) and Early White Bush Scallop and Zucchine pumpkins (*Cucurbita pepo* L.), potato tubers (*Solanum tuberosum* L.), and roots of garden beet (*Beta vulgaris* L.), turnip (*Brassica rapa* L.), hollyhock (*Althea rosea* Cav.), cotton (*Gossypium hirsutum* L. var. Acala), Chinese hibiscus (*Hibiscus rosa-sinensis* L.), and carrot (*Daucus carota* L. var. *sativa* DC.) were unsuccessful. Dowson (*3*), using an isolation from rotted carrots, was unable to secure infection through unwounded surfaces. Potato tubers inoculated by placing mycelium and oospores in a small slit about 4 mm deep were invaded by the isolate from cineraria and by Ashby's culture. The type of infection was similar to that resulting from infection by numerous species of *Phytophthora*, the infected tissues becoming pink on exposure to the air. The isolates from stock, cabbage, hollyhock, and cauliflower did not cause infection. Similarly, inoculated apple fruits were infected by all isolates, with the development of a light brown, mealy type of decay.

SUMMARY

A root rot of cauliflower, caused by *Phytophthora megasperma* Drechsler, is responsible for losses in the winter cauliflower crop in the coastal districts of California. The disease occurs only where the soil has become waterlogged.

The disease also occurs on cabbage, Brussels sprouts, cineraria, and stock.

The outer leaves of infected cauliflower plants show a reddish discoloration and later the plants wilt rather suddenly. Affected plants are easily pulled, and the basal end of the taproot is found to be rotted to such an extent that the cortex usually sloughs off.

The cauliflower fungus is described and compared with isolates from certain other hosts.

Infection was obtained by adding the fungus to the soil of potted cauliflower plants and incubating them outdoors in buckets containing water about 4 inches deep. The incubation period was about 3 weeks. Infection was also obtained on stock, cineraria, and wallflower plants and on ripe tomato fruits. Attempts to infect certain other unwounded vegetables were unsuccessful.

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A SUBIRRIGATION METHOD OF SUPPLYING NUTRIENT SOLUTIONS TO PLANTS GROWING UNDER COMMERCIAL AND EXPERIMENTAL CONDITIONS [1]

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METHODS OF OTHER INVESTIGATORS

Various methods have been reported in the literature for the growing of plants on a large-scale basis under controlled nutritional conditions. Gericke (4)[2] reports satisfactory results when nutrient solutions are used in shallow tanks or ponds. The plants are supported on a wire netting which is suspended at the surface of the solution. A seedbed is prepared by covering the netting with burlap and a ½-inch layer of sand. The seeds germinate in the moist sand and the roots grow through the bed into the nutrient solution. The nutrients are supplied in cartridges which slowly dissolve.

The use of an inert medium, such as sand, greatly simplifies the problem of support and aeration. In the so-called slop culture technique (1, 2), the sand is put into well-drained benches or crocks and the nutrient solution poured on at intervals, the loss of water by transpiration being supplied by watering the surface of the sand. The main disadvantage of this method is that the nutrients are supplied too infrequently to insure reasonably constant osmotic concentration or balance of ions in the vicinity of the absorbing regions of the roots. Commercially, the system is wasteful of nutrients and requires more labor in watering and supplying nutrients than does soil culture.

By supplying a constant flow of solution through the sand, Shive and Stahl (5) were able to maintain relatively constant nutritional conditions around the roots of plants in sand culture under actively growing conditions. The method is now commonly known as the "drip culture" technique and is widely used in critical studies in plant nutrition. Crocks of sand are usually employed, the nutrient solution being supplied from a separate reservoir for each crock. The solution drips at a constant rate from a capillary tube into a modified thistle tube so that air is trapped with the solution. The method, however, does not lend itself to large-scale production because of its complexity and wastefulness of nutrients. Żurbicki (7) has developed an automatic method of drip culture for supplying the nutrients intermittently rather than by continuous drip. According to this method, the whole mass of sand is bathed on each cycle, and the process may be repeated as frequently as desired.

The sand-culture system described by Eaton (3) lends itself to larger scale production with less manipulation than the drip-culture method. It consists essentially of shallow tanks of sand fitted with perforated drainage pipes in the bottom and perforated solution

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supply lines on the surface of the sand. The solution flows from an elevated tank through the sand into a lower tank so that the sand is thoroughly flushed at each operation. The solution in the lower tank is forced into the upper tank by reduced air pressure, maintained by a filter pump. One air pump may be used to supply a series of independent systems containing different solutions.

THE SUBIRRIGATION METHOD

The present system was developed to combine the advantages of the uniform supply of nutrients and aeration of the drip-culture technique with the relative simplicity of the slop-culture method without the attendant waste of nutrients of either system. The method has the added advantage of being capable of automatic operation over long periods of time. In principle, it consists in supplying the nutrient solution through a perforated pipe laid in the bottom of a watertight shallow bed, such as a greenhouse bench, which is filled with coarse sand, fine gravel, or cinders. The pipe is connected to a centrifugal power-driven pump which operates at intervals determined by a time clock, for a period sufficiently long barely to flood the medium, after which the clock opens the pump-motor circuit. The solution flows back through the pump by gravity into the reservoir. By the proper choice of media, the cycle may be repeated as frequently as desired, so that the roots are continuously flushed with air and nutrient solution. The essential features of the system are shown diagrammatically in figure 1.

The watertight beds may be constructed very economically by the application of asphaltic roofing materials to existing greenhouse ground beds and raised benches. Roofing asphalt is largely derived from petroleum distillation and appears to be relatively free from water-soluble toxic substances. Roofing tars, on the other hand, are mainly derived from coal distillation and contain tar acids which, together with other soluble substances, may cause severe plant injury.

All roofing asphalts are not entirely of petroleum derivation and may be modified with tars. It is wise, therefore, to find out from the manufacturer the composition of the asphaltic materials before using them in waterproofing benches. Petroleum asphalts may be obtained directly from the manufacturers of petroleum products as air-blown asphalts.

Concrete benches are easily waterproofed by painting with a cut-back asphalt (asphalt dissolved in a volatile solvent, such as naphtha), with asphalt roofing paint, or by mopping with hot fluid asphalt. The successful application of hot asphalt depends upon its being heated to a high enough temperature. Three hundred and seventy-five to four hundred degrees Fahrenheit is generally considered the correct temperature range for laying built-up roofs and is not far from the flash point, so that considerable care must be exercised.

In the construction of new benches, the concrete may be water-proofed by the addition of special asphalt preparations which are available for the purpose. The use of these preparations eliminates the necessity of surface treatment. Concrete benches are especially adaptable to the subirrigation system because the bottom of the benches may be sloped toward the center or the sides in such a way as to insure more complete drainage than could be obtained with a

level bottom. Such a sloping construction is more difficult in the case of a wooden bench.

For wooden benches an asphalt roofing paper may be used. It is most inexpensively obtained in the form of a plain felt. The rag felts rot out easily unless given a heavy coating of asphalt. The asbestos felts, while somewhat more expensive, are superior in strength
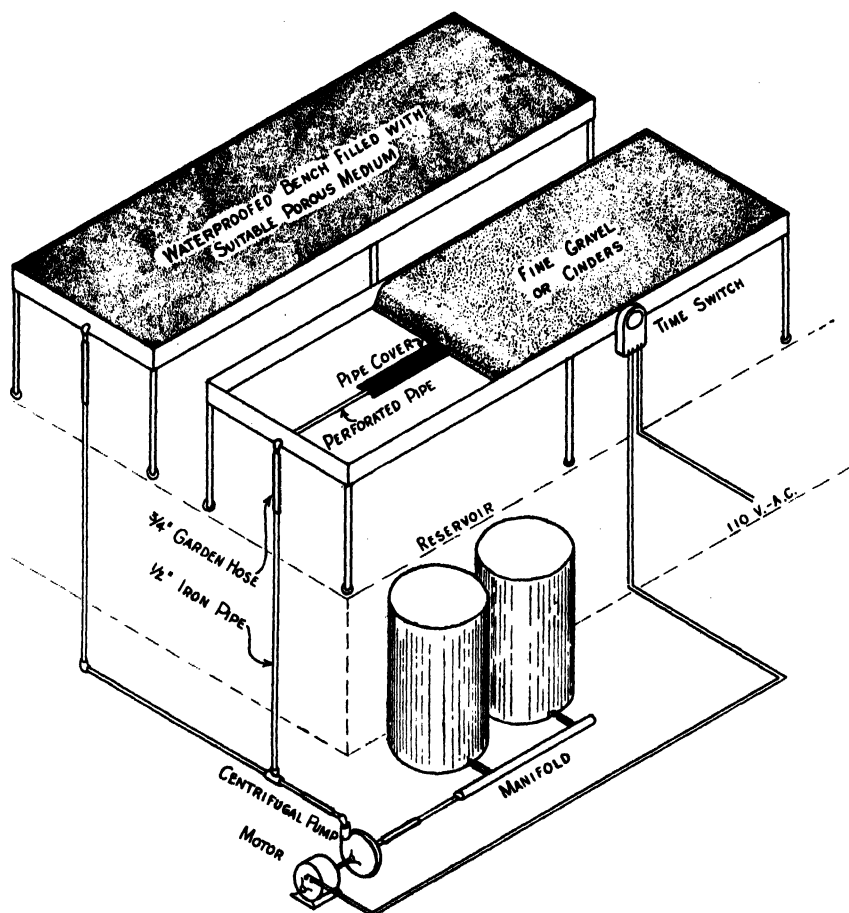
FIGURE 1.—Diagrammatic view of the subirrigation system for large-scale operation.

and decompose very slowly. In one instance, an uncoated asphalt-bound rag felt rotted so badly in 2 months that severe injury of the roots of the plants resulted from the production of hydrogen sulphide and possibly other substances of a toxic nature.[3]

The felt is so laid on the bottom of the bench and folded up over the sides as to form a complete lining. A single ply of 20-pound asbestos felt, hot-mopped on the upper surface with asphalt, is not

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[3] E. F. Ries, of the Johns-Manville Corporation, recently informed the authors that tests carried on in connection with the wrapping of buried oil lines showed that, after a 15-pound asphalt-saturated rag felt had been buried for 209 days, its tensile strength decreased from 52.4 to 8.7. A similar test with an asphalt-saturated asbestos felt decreased from 39.4 to 33.2. Soil-mulching experiments with tar- and asphalt-saturated felts indicated that tar is toxic to plants while asphalt is not.

strong enough for more than a temporary set-up. For permanent waterproofing, two- or three-ply construction is necessary, hot-mopped between each layer of felt in exactly the manner of constructing built-up roofs. The most vulnerable points in the bench are the bottom edges where the felt folds up along the sides. Here the felt is weakly supported, and a 1- by 1-inch flashing strip of triangular section materially aids in preventing the tearing of the felt layers. The seams are cemented with an asphaltic asbestos roof putty. After the putty has dried, it is well to give the whole surface a coating of asphalt, using a roofing paint, or, better still, an application of hot asphalt as described for concrete benches. Coating with sand or fine gravel while the surface is still tacky is desirable.

The pipe for supplying the nutrient solution is laid on the bottom of the bench, running straight through the end boards. One end is capped so that the pipe may be flushed out in case fine sand or silt works in. The pipe is drilled with ¼-inch holes spaced 12 inches apart on the side laid next to the bottom of the bench. A 4- to 6-inch strip of heavy prepared roofing or painted felt is laid over the pipe and should run the full length. This narrow strip very effectively prevents fine material from working into the holes in the pipe line and clogging it up. A thin layer of sand or gravel will keep the strip from sticking to the bottom of the bench and will prevent interference with the flow of solution.

Black-iron pipe is useful for experimental work, since it has no toxic effect on the plants. However, it has a tendency to rust under the well-aerated conditions present. Galvanized-iron or copper pipe might be satisfactorily used. Good growth resulted in a preliminary test with copper tubing when plants were grown on a small scale, but a large-scale test has not been tried by the authors. The copper inhibited the growth of algae, which fact may in itself be an advantage. No tests have been made with galvanized-iron pipe. One pipe line is sufficient for a bench 4 to 5 feet wide filled with a fairly coarse medium. A ½-inch pipe has proved adequate for a 50-foot length of bench, with the intake at one end only. For longer benches, it would probably be necessary to use larger pipe with holes spaced closer together at the far end from the intake.

A motor-driven centrifugal pump is used to force the solution up into the beds. When the motor circuit is opened, the solution drains back through the pump into a reservoir. A ¼-horsepower pump will handle 2,500 square feet of bench filled with 5 to 6 inches of fine gravel, and less than half an hour will be required to flood the benches when the bottom of the reservoir is 4 feet below the bench. Fifty-gallon alcohol or thoroughly cleaned oil drums connected through a manifold make very useful reservoirs for the amount of solution necessary for small areas. With fine gravel or properly sifted cinders, it may generally be considered that the reservoir capacity is at least one-fourth the volume of the medium in the bench. On this basis, 1,000 square feet of bench space containing 5 inches of medium would require approximately 110 cubic feet or 800 gallons as the minimum reservoir capacity. The amount necessary varies a great deal depending upon range of particle size and proportion of coarse to fine material.

In an experimental installation, very convenient flexible connections may be made to the tanks, pump, and benches with short lengths of ¾-inch rubber garden hose slipped onto ½-inch iron pipe. Such a method of coupling does not require accurate pipe fitting and is quite easy to install.

An electric time switch set for three half-hour intervals per day is being used by the authors to control the operation of the pump. Such a system involving 1,000 square feet of bench space has operated automatically without any attention whatsoever for a week or more and the plants have remained in good condition throughout the period, being supplied with nutrients at regular intervals. The interval of feeding is largely dependent upon the nature of the experiment, the medium in the bench, the size of the plants, and the season of the year.

The most critical feature of the system is the minimum-particle size of the medium supporting the roots. Any but the finest sand will allow water to flow freely through it. However, when the roots stand in a shallow, drained bench, there may be few or no air spaces left after drainage, owing to the presence of water held by capillarity. During periods unfavorable for transpiration, as in winter, a longer time is required for the water to be transpired, and root injury due to lack of oxygen may result if the pumping cycle is repeated daily or more often. Repeating the cycle less frequently results in nonuniform nutritional conditions.

It is, therefore, essential for the satisfactory operation of a sub-irrigation method of nutrient supply that the supporting medium be sufficiently coarse to allow the solution to drain out fairly completely and be replaced with air after each cycle of operation. In general, it has been found advisable to remove by washing the great bulk of particles which will pass through a 16- to 20-mesh sieve (1.6 to 1.2 mm). This practically eliminates sand as a satisfactory medium, except under conditions favoring high transpiration. Fine gravel having particle sizes ranging from ⅟₁₆- to ¼-inch in diameter appears to be the most suitable for this type of culture.

The relatively inert neutral or acid gravels can be used with any of the common nutrient solutions without having much effect chemically, except possibly to supply traces of minor elements. Cinders, which have been thoroughly leached and sifted, fall in the same class. Since cinders are very porous, the upper limit of particle size may satisfactorily be in the vicinity of one-half inch for woody plants, such as rose or gardenia.

If the cinders are allowed to weather for a winter and then sifted, they may be used directly in the bench with no more than one or two thorough washings. New cinders have been used without any noticeably toxic effects by allowing water to stand for a day at a time on the benched medium. Three or four days of such washing, using clean water each time, will remove most of the soluble substances.

Calcareous gravels containing appreciable quantities of calcium carbonate have a decided influence on the nutrient solution and frequently it may be necessary to modify the solution considerably. The authors have used a fine calcareous gravel containing about 20 percent of acid-soluble material, largely calcium carbonate. In the absence of sufficient phosphate, the gravel buffers the nutrient solution in the region

of pH 8. When, however, the gravel is washed with a dilute solution of soluble phosphate to which is then added the nutrient solution, the buffering action takes place at pH 6.6 to 6.8. Possibly the surfaces of the calcium carbonate particles become coated with a mixture of calcium phosphates, which results in a buffering action at a lower pH value.

A pH value of 6.6 is quite satisfactory for many plants, but not for such plants as gardenia and some roses, which tend to become chlorotic. Attempts to increase the acidity with a soluble acid phosphate or sulphuric acid were relatively ineffective since the acid kept bringing more calcium into solution. If limestone is present in the system at all, it does not appear possible to maintain pH values much below 6.6 until it is all dissolved. This is a very important consideration when cinders or neutral or acid sands are used at low pH values. In one instance, the presence of a small amount of calcareous furnace lining in a load of cinders caused considerable difficulty by constantly raising the pH value until all of it had been dissolved with sulphuric acid.

In some sections, the readily available gravel and sand contain appreciable quantities of calcium carbonate. Such is the case in the locality in which the present investigation was made. Cinders were tried as a substitute, and thus far, they have proved, quite satisfactory for the larger woody plants. The particles are rather sharp and coarse, however, for soft herbaceous seedlings. In experimental set-ups where it is desired to obtain root weights, cinders should not be used because the particles are sharp and it is almost impossible to wash them out from the fine roots.

The element of cost becomes an important item in the use of pure chemicals when it is desired merely to get good growth. The usual three- and four-salt nutrient solutions may be readily modified to use commercial fertilizer chemicals which are inexpensive and which usually contain sufficient traces of the minor elements. The monopotassium phosphate in the Shive solutions (6) may be replaced with equimolarities of monocalcium phosphate as triple superphosphate and potassium sulphate. Calcium nitrate, potassium nitrate, ammonium sulphate, ammonium nitrate, ammonium phosphate, and calcium phosphate (superphosphate) are all available as fertilizer salts. Sufficient magnesium sulphate may be supplied through the use of the double salt potassium magnesium sulphate. This salt is available as a commercial fertilizer and is very useful as a source of potassium and magnesium. With these crude salts and their approximate analyses, it is possible to practically duplicate any of the usual nutrient solutions made up on a three- or four-salt basis as far as securing good growth is concerned.

The foregoing discussion has dealt mainly with large-scale production in which whole benches are supplied with the same nutrient solution by means of a power-driven pump. Small-scale experiments requiring the use of several different solutions may be set up with 18-liter bottles for reservoirs and gravity as a means of forcing the solution into the media and draining it. The bottles are made self-draining by wiring an inverted U-shaped piece of ⅛-inch black-iron pipe to the side of the bottle. A ⅜-inch rubber hose is attached to the outside end of the pipe and connected with greenhouse flats. The flats are lined with roofing paper as previously described and a perforated piece

of ¼-inch iron pipe is placed on the bottom, over which is laid a 3-inch strip of roofing paper. The ¼-inch pipe is cut 2 inches longer than the outside dimensions of the flat so that it runs through both end boards and is capped at one end. The other end connects with a short piece of ⅛-inch pipe through a reducer. Three-eighth-inch rubber tubing fits snugly on ⅛-inch standard iron pipe.

The circulation of nutrients is accomplished by raising the bottles, as shown in figure 2, until the medium containing the plants is barely flooded. The bottles are then lowered and the solution drains back. Repeating the process one to three times a day insures a relatively constant supply of nutrients to the roots and provides thorough aeration. If crocks or hard-glass beakers are used, a similar system
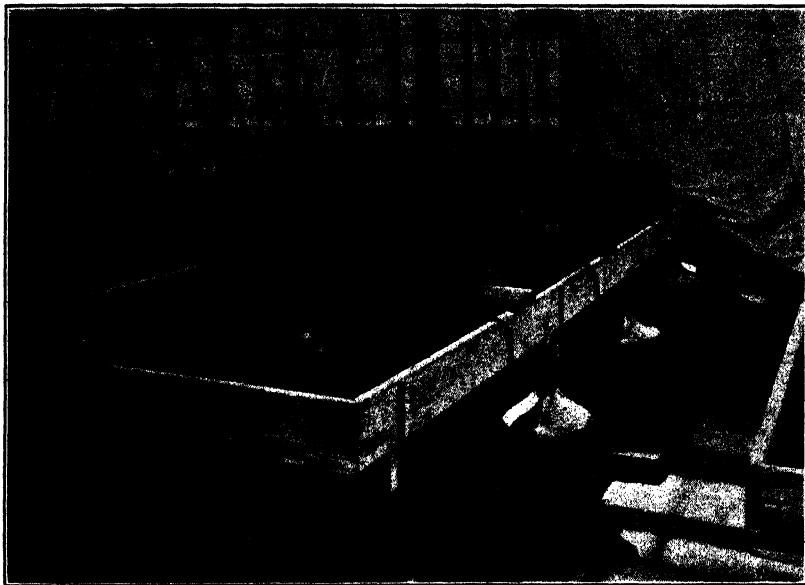
FIGURE 2.—Four pairs of flats (3½ by 15 by 22 inches) fed with 4 separate nutrient solutions in 18-liter bottles. The siphon is maintained by inverted U of ⅛-inch black-iron pipe. The flats are waterproofed with asphalt roofing paper. In the bottom of each flat is a perforated pipe over which is placed a 3-inch strip of heavy roofing paper to keep out fine material.

may be employed involving only glass, quartz sand, and rubber tubing. In this case, a siphon is used in the beakers of sand also.

Figure 3 shows a similar set-up in which the plants are growing in individual containers. The buckets are painted on the inside with asphalt paint and supplied with perforated tubing covered with a disk of asphalt roofing. The nutrients are supplied from a 40-liter crock suspended by a pulley arrangement. By the use of counter-weights, the crock may be lowered and raised with relative ease. When several buckets or flats are connected together, one will drain first and sometime break the siphon. A second return of small tubing dipping into the top of the reservoir will satisfactorily drain the system. The advantage of growing plants in individual con-tainers is that it enables the experimenter to remove any container desired at any time for special treatment.

SUMMARY

A subirrigation method of supplying nutrient solutions to plants growing in sand culture which lends itself to large-scale production is described. It consists in principle of pumping nutrients from a submerged reservoir into the bottom of a shallow bed of fine gravel or cinders with a centrifugal pump. The pump is controlled by an electric time switch which stops the operation when the bench is flooded. The solution then flows back into the tank through the pump by gravity.
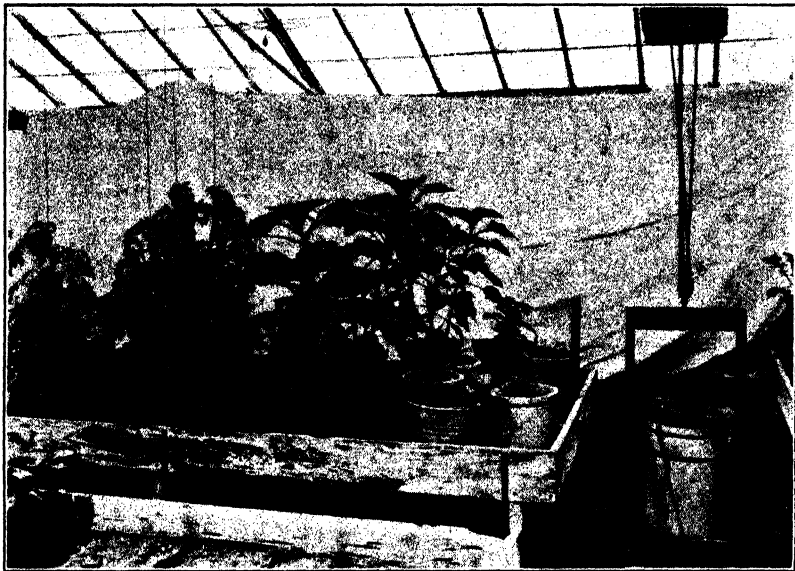
FIGURE 3.— A series of buckets and crocks fed with nutrients from a 40-liter crock which is raised and lowered by a counterbalanced system of pulleys. The solution flows in through a ¾-inch hose connected to a ½-inch iron manifold to which is soldered ⅛-inch iron pipe for supplying the containers of fine gravel. The small tubing allows the solution to drain back in case air should break the siphon.

Modifications of the method are discussed for use in experimental set-ups on a small scale, in which the solution flows into and out of the plots by gravity through the raising and lowering of bottles of solution.

. The advantages of the system are (1) frequent and complete flushing of the roots with air and nutrient solution, (2) economy of nutrients since the solution drains back into the tank again, and (3) completely automatic operation over long periods of time.[4]

[4] Since the foregoing manuscript was submitted for publication several improvements in the method have been made.

Wooden benches may be very permanently waterproofed with hot asphalt and gravel. The cracks in the bench are lightly covered with lath and the bottom leveled off with a thin layer of fine gravel. Asphalt heated to 400° F. is then applied over the whole surface with a sprinkling can. At this temperature the asphalt flows readily through the fine holes of a nozzle. A second layer of gravel and asphalt is then applied. One and one-half to two pounds of asphalt is required per square foot. The dry, tamped soil of ground beds has been satisfactorily treated in the same manner. Six-inch boards are used as sides. The nutrient solution may be more uniformly supplied to the bench by substituting for the perforated iron pipe and cover a single line of inverted 4-inch double-beaded galvanized roofing gutter laid on the bottom of the bench so as to form a large open channel for the unimpeded flow of solution. A short piece of ½-inch pipe sealed in the end of the bench admits the solution. Before the gutter is installed it should be given several coats of asphalt roofing paint to keep the solution from direct contact with the zinc surface, which has been found to be toxic to plants in highly acid solutions. Such a large open channel is easier to install than pipe and shows little tendency to clog with roots.

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ROLE OF INSECTS IN THE DISTRIBUTION OF COTTON WILT CAUSED BY FUSARIUM VASINFECTUM [1]

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INTRODUCTION

The methods of spread of cotton wilt caused by *Fusarium vasinfectum* Atk. have received considerable attention from investigators. It has long been suspected that insects frequently play a part in the local spread of cotton wilt. During 1933 and 1934 the writers sought to obtain definite information on the possibility of spread of wilt through the fecal pellets of insects that normally feed on the different parts of cotton plants known to be infected with fusarium wilt. Some attention was also given to spread of *F. vasinfectum* as adhering spores on the exterior parts of the body of cotton insects. The laboratory studies here reported were made at College Station, Tex. The field data were obtained in cotton fields near College Station, Beaumont, and Caldwell, Tex. Brief reports on this work appeared in 1933 and 1934.[2]

METHODS AND MATERIALS

The experiments were divided into three groups dealing with cotton insects feeding on (1) roots, (2) stems and leaves, and (3) bolls. The few species worked with were selected because of the ease with which they could be submitted to experimental conditions.

In the laboratory, field-collected insects were caged with portions of roots, stems, or leaves of cotton plants known to be infected with *Fusarium vasinfectum*. The fecal pellets from these insects were collected at least once a day and dried so that they would not quickly disintegrate when placed in fluids. Then they were surface-sterilized by being placed for 30 seconds in an aqueous solution containing, by weight, 0.05 percent mercuric chloride and 35 percent ethyl alcohol, and finally, after being rinsed three times in sterile water, they were planted in Petri plates on acidified potato-dextrose agar. In other tests, certain cotton insects were allowed to feed on normal cotton leaves that had been sprayed with a heavy suspension of spores of a pure culture of *F. vasinfectum*. The fecal pellets from such insects were collected each morning and cultured in the same manner. From time to time insects were dissected under aseptic conditions, and fecal pellets about to be defecated were extracted and cultured. In other tests, insects were permitted to feed on wilt-infected cotton material, and then, after prolonged surface sterilization, the entire insects were cultured on nutrient agar in Petri dishes.

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In field tests designed to determine whether cotton insects act as carriers of *Fusarium vasinfectum*, a number of insects were collected in wilt-infected fields. Where possible they were picked up with sterile forceps. To obtain very active species it was necessary to net them or bat them down with a swatter. Each individual thus secured was placed in a sterile vial plugged with cotton and brought to the laboratory, surface-sterilized, and then cultured, or fecal pellets about to be defecated were dissected out and cultured. Soil-inhabiting insects in wilt-infested cotton fields were collected by means of a Berlese funnel and then surface-sterilized and cultured.

Wherever possible, precautions were taken to prevent or minimize casual contamination of fecal pellets or insects to be cultured. All cages and vials were heat-sterilized before use. In experiments with white grubs the soil in which the insects were confined was heat-sterilized at intervals. Spore-covered leaves used for the leaf-feeding insects were suspended at the tip of vials to prevent falling fecal pellets from coming in contact with the leaf areas.

EFFECTIVENESS OF STERILIZATION METHOD

It was established early in these experiments that an aqueous solution containing, by weight, 0.05 percent mercuric chloride and 35 percent ethyl alcohol was effective in destroying the hyphae, chlamydospores, and the microspores as well as the macrospores of *Fusarium vasinfectum*. This solution, however, did not always spread uniformly over the entire surface of fecal pellets or of entire insects when these were characterized by deep sutures or by hirsute bodies or appendages.

Tests to determine the effectiveness of surface sterilization were made by covering entire insects and fecal pellets with a spore suspension of *Fusarium vasinfectum*. One lot each of larvae and fecal pellets of *Alabama argillacea* Hbn. thus treated was surface-sterilized with the mercuric chloride-alcohol solution with 100-percent effectiveness, while in a lot of grasshoppers similarly treated sterilization was only 74 percent effective. Results of the many experiments subsequently made, however, indicated that in the main this sterilization method was highly effective. Nevertheless, external sterilization alone was not relied on. The results were checked from time to time by culturing fecal pellets dissected out from the recta of insects under aseptic conditions.

IDENTITY AND PATHOGENICITY OF THE FUSARIA

All isolations of the fusaria in these experiments were finally grown in tubes on potato-dextrose agar or on sterilized rice. Morphologically, and in pure culture, the strains of *Fusarium* recovered from the fecal pellets or insects used in these tests could not be distinguished from typical *F. vasinfectum* isolated from the roots or stems of a naturally infected cotton plant. Many of these strains were identified by Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station, as *F. vasinfectum*, but somewhat different from *F. vasinfectum* that affects cotton in Tennessee. Cotton seedlings were successfully inoculated with pure cultures of *F. vasinfectum* obtained from Sherbakoff. Successful inoculations were also made with strains of *Fusarium* recovered by the writers at College Station and Beaumont from insects that had fed on normally infected cotton

plants and from the fecal pellets of these insects. It is of interest to note that more than 80 percent of the fusaria recovered from the fecal pellets of the cotton insects used in these experiments were the same as or similar to *F. vasinfectum*. Other organisms recovered, particularly from the fecal pellets, were *F. semitectum* and various species of *Aspergillus*.

In November 1934 several thousand fecal pellets were collected from grasshoppers that had fed on cotton stems naturally infected with cotton wilt. These pellets were kept at room temperature, in a test tube plugged with cotton, and cultures were made of them from time to time. The percentage of pellets from which *F. vasinfectum* was recovered is shown in table 1. It will be noted that *F. vasinfectum* remained viable for at least 15 months.

TABLE 1.—*Viability of Fusarium vasinfectum in dried fecal pellets obtained November 1934 from grasshoppers fed on wilt-infected cotton stems*

Date cultured	Pellets cultured	Pellets from which *F. vasinfectum* was obtained	
	Number	*Number*	*Percent*
December 1934	160	147	91.9
April 1935	210	180	85.7
November 1935	117	93	79.5
March 1936	287	117	40.8

RECOVERY OF FUSARIUM VASINFECTUM FROM ROOT-FEEDING INSECTS

The root-feeding insects used included white grubs (*Phyllophaga crassissima* Blanch. and other unidentified species), wireworms, and numerous small insects usually included in the category of "smaller soil animals."

The white grubs were placed in soil cages and fed on portions of cotton roots infected with *Fusarium vasinfectum*. The fecal pellets were collected daily by soil sifting and cultured in the usual way. As shown in table 2, out of 239 fecal pellets cultured, 78, or 32.6 percent, yielded pure cultures of *F. vasinfectum*. Likewise, cultures were made of 17 entire white grubs that had been feeding on infected cotton roots, and 4, or 23.5 percent, yielded pure growth of *F. vasinfectum*.

TABLE 2.—*Petri-dish cultures of fecal pellets of white grubs (Phyllophaga crassissima and others) fed on portions of cotton roots infected with fusarium wilt*

Degree and type of feeding	Fecal pellets cultured	Cultures showing good growth of *Fusarium vasinfectum*	
	Number	*Number*	*Percent*
Slight to moderate surface grazing	17	1	5.9
	1	1	100.0
	16	16	100.0
	3	2	66.7
	3	0	0.
	3	0	0.
Moderate gouging	11	5	45.5
	12	0	0.
	12	8	66.7
	119	20	16.8
	17	17	100.0
Copious gouging	25	8	32.0
Total	239	78	32.6

A number of wireworms were placed in soil cages and permitted to feed on the roots of wilt-infected cotton plants. After feeding, they were surface-sterilized and the entire insects cultured. In not a single instance was *Fusarium vasinfectum* recovered.

Among the smaller soil animals worked with were the Collembola (including *Onychiurus fimetarius* (L.) Lubb., *Pseudosinella violenta* Fols., and *Entomobrya sabulicola* Mills [3]), a species of Japygidae, and coleopterous larvae. They were collected from the soil about the roots of wilted cotton plants and cultured with and without surface sterilization. Certain of the Collembola, having shown a marked avidity for mycelial growth of *Fusarium vasinfectum*, were fed for from 3 to 24 days on pure slant cultures of the cotton-wilt fungus. General collections of smaller insects obtained from the soil surrounding wilt-infected cotton roots were surface-sterilized and also cultured. The results are shown in table 3. It will be noted that, of all the specimens of Collembola cultured, only one (*P. violenta*), which was not surface-sterilized, yielded growth of *F. vasinfectum*. Growth of *F. vasinfectum* was obtained from two of four unidentified coleopterous larvae cultured. In cultures of approximately 1,000 smaller soil insects and other Arthropoda, 6 yielded growth of *F. vasinfectum*.

TABLE 3.—*Petri-dish cultures of smaller soil animals collected from soil under wilted cotton plants or fed upon pure cultures of Fusarium vasinfectum prior to culturing*

Species	Source or treatment of insects	Preparation for culturing	Insects cultured	Insects yielding growth of *Fusarium vasinfectum*	
			Number (¹)	*Number*	*Percent*
Onychiurus fimetarius	Soil under wilted plant	Not sterilized	7	0	
	---do---	---do---		1	14.3
Pseudosinella violenta	---do---	Washed and sterilized	17	0	
	Fed on *F. vasinfectum*	Washed in sterile water	4	0	
	---do---	Washed and sterilized	15	0	
	Soil under wilted plant	---do---	2	0	
Entomobrya sabulicola	Fed on *F. vasinfectum*	Not sterilized	7	0	
	---do---	Washed in sterile water	4	0	
	---do---	Washed and sterilized	18	0	
Japygidae	Soil under wilted plant	Not sterilized	5	0	
Coleopterous larvae	---do---	Washed and sterilized	4	2	50.0
Miscellaneous smaller soil animals, etc.[²]	---do---	---do---	[3] 1,000	6	.6

¹ Several.
² Composed of Collembola, Japygidae, Campodeidae, larval stages, etc. Other arthropods, such as Pauropoda, Symphyla, Diplopoda, Chilopoda, Acarina, Araneida, etc., were included.
³ Approximate number.

RECOVERY OF FUSARIUM VASINFECTUM FROM STEM- AND LEAF-FEEDING INSECTS

The stem borer *Ataxia crypta* Say [4] is frequently found infesting dead stems of cotton plants in Texas and consuming tissues normally attacked by the cotton-wilt fungus. In feeding within cotton stems this insect usually expels considerable frass through a small hole maintained for the purpose. The frass falls to the ground or is blown away by the wind. A number of *A. crypta* were introduced into stems of cotton plants that had been killed by *Fusarium vasinfectum*. Nine entire larvae, fecal pellets, and frass were surface-

³ Identified by J. W. Folsom, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.
⁴ Identified by H. J. Reinhard, Texas Agricultural Experiment Station.

sterilized and cultured in the usual way. *F. vasinfectum* was recovered from the frass and from six of the nine entire larvae.

Among the grasshoppers [5] commonly found in wilt-infected cotton fields in Texas are the following: *Melanoplus differentialis* Thos., *M. mexicanus* Sauss., *M. femur-rubrum* Deg., *Encoptolophus texensis* Brun., *Spharagemon cristatum* Scudd., *Tomonotus aztecus* Sauss., *Chortophaga viridifasciata* var. *australior* Deg., *Schistocerca americana* Dru., *S. obscura* Fab., *Trimerotropis citrina* Scudd., and *Dissosteira carolina* L.

A number of grasshoppers of the species *Melanoplus femur-rubrum*, *M. differentialis*, and *Schistocerca obscura* and an unidentified miscellaneous group were caged and permitted to feed on the stems of wilt-infected cotton plants.   The insects were forced to consume diseased tissues or starve, inasmuch as all foliage had been removed. The fecal pellets were collected each morning and cultured immediately after surface sterilization.   As shown in table 4, 25 lots containing a total of 1,729 fecal pellets, were cultured.   Pure growth of *F. vasinfectum* was recovered from 80 percent of these lots.   In addition, four surface-sterilized adult grasshoppers were cultured, one of which produced a growth of *F. vasinfectum* originating at a point on a tarsus.

TABLE 4.—*Petri-dish cultures of fecal pellets of grasshoppers fed on portions of cotton stems infected with fusarium wilt*

Species	Fecal pellets cultured	Growth of *Fusarium vasinfectum* [1]
	Number	
	36	−
	59	+
	56	+
	42	−
	85	+
	50	−
	39	+
Melanoplus differentialis	51	+
	40	+
	49	+
	100	+
	100	+
	72	+
	149	+
	164	+
	50	+
Melanoplus femur-rubrum	205	+
	18	+
Schistocerca obscura	12	+
	44	+
	57	−
	46	−
Miscellaneous [2]	55	+
	100	+
	50	+
Total	1,729	5 −, 20 +

[1] + denotes positive growth of *F. vasinfectum*, − no growth.
[2] Chiefly *Melanoplus differentialis*; also *M. femur-rubrum* and unidentified nymphs.

During the season of 1934 field studies were made to determine the extent to which leaf-feeding insects in badly diseased cotton areas may act as carriers of cotton wilt.   Grasshoppers, including all the

[5] Identified by the late A. N. Caudell, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

species mentioned above, were used in these experiments, because they are active fliers, are prevalent in wilt-infected cotton fields in Texas, and are also known to consume quantities of cotton-leaf tissue. Pure cultures of *Fusarium rasinfectum* were obtained from 22.2 percent of 419 grasshoppers collected near Caldwell, from 26.6 percent of 184 collected near College Station, and from 33.3 percent of 90 obtained near Beaumont. In the Caldwell field, from which data of a seasonal nature were obtained, 9.4 percent of the grasshoppers were found to carry the wilt fungus internally on July 24, 11.7 percent on August 7, 35.6 percent on September 4, 24.9 percent on September 27, and 29.5 percent on October 18. These statements apply only to those species of grasshopper for which positive results were obtained. Several other species that were present in the fields in smaller numbers were not tested.

There was no way of determining whether the number of grasshoppers acting as carriers of *Fusarium rasinfectum* was in direct ratio to the number of plants infected in the field. All fields in which surveys were made proved ultimately to have 75 percent or more of the plants diseased.

Larvae of *Alabama argillacea*, *Laphygma frugiperda* S. and A., and *Prodenia ornithogalli* Guen. obtained from nonwilt areas were placed in separate cages and fed on cotton leaves naturally infected with wilt. In addition, larvae of *Alabama* and *Prodenia* were fed on leaves that had been painted with a heavy suspension of spores of a pure culture of *Fusarium rasinfectum*. The fecal pellets and some of the entire insects were cultured in the usual way. As shown in table 5 and figure 1, typical *F. rasinfectum* was often recovered from the fecal pellets of the larvae and from some of the entire insects.

TABLE 5.—*Petri-dish cultures of fecal pellets and various stages of lepidopterous leaf-feeding insects*

Species	Kind of food consumed	Material	Cultures	Cultures showing good growth of *F. rasinfectum*	
			Number	Number	Percent
Laphygma frugiperda	Naturally infected cotton leaves.	Fecal pellets	5	0	0
			29	16	55.2
			40	0	0
			114	43	37.7
			8	8	100.0
Prodenia ornithogalli	....do....	...do...	1,826	559	30.6
	....do....	Entire larvae	20	1	5.0
	Spore-covered cotton leaves.	Fecal pellets	52	29	55.8
Alabama argillacea	Naturally infected cotton leaves.	...do...	768	615	80.0
	....do....	Entire larvae	9	2	22.2
	Spore-covered cotton leaves.	Fecal pellets	202	202	100.0
	....do....	Entire larvae	2	0	0
	....do....	Prepupae	1	0	0
	....do....	Pupae	10	1	10.0
	....do....	Adults	4	0	0

Tests were made to determine whether the wilt organism could remain within *Alabama argillacea* during pupation and later be disseminated by the adult moth, which has exceptional migratory powers. This problem was attacked by determining the degree of completeness with which the alimentary tract was voided of viable fungus material through the elimination processes. Larvae were fed

for a time upon normal cotton leaves, then upon spore-painted leaves, and then once more upon normal leaves. Fecal pellets were cultured individually as defecated. No growth of *Fusarium vasinfectum* was obtained until approximately 26 minutes after spore-covered cotton leaves had been provided as food. All fecal pellets were then found to sponsor growth of the fungus until approximately 26 minutes from the time the second feeding on normal leaves had begun. Thereafter no growth of *F. vasinfectum* was obtained. In other tests larvae were allowed to feed on spore-covered cotton leaves and then starved for
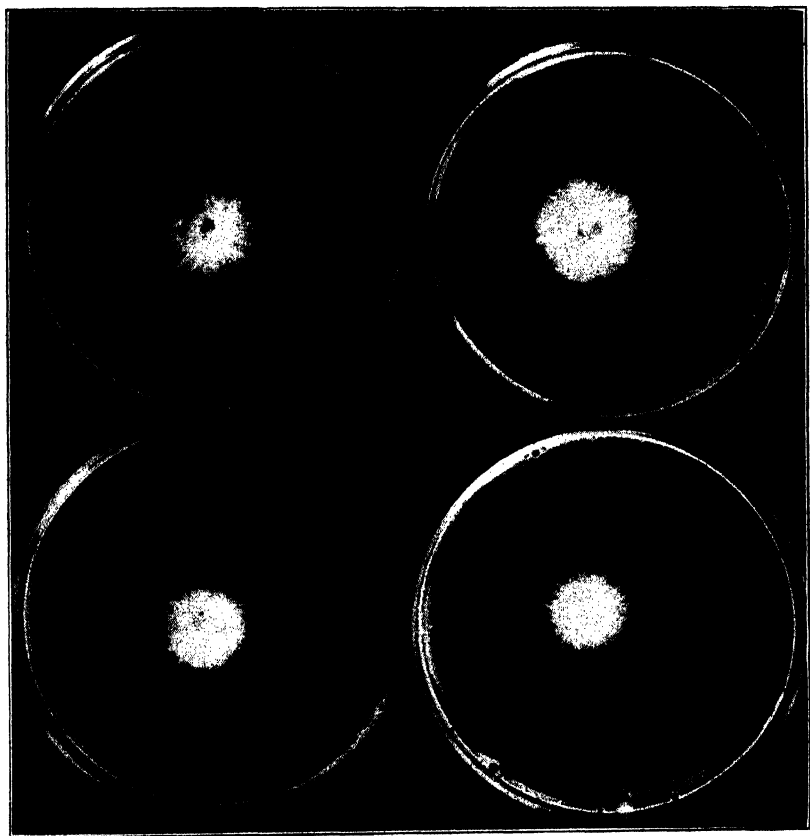
FIGURE 1.—Pure cultures of *Fusarium vasinfectum* recovered from individual fecal pellets of *Alabama argillacea* larvae that had been fed on cotton leaves painted with a suspension of the spores of *F. vasinfectum*.

from 1 to 4 days. These larvae were then dissected and their alimentary tracts aseptically removed and cultured. The results were always negative.

Similar tests were made with *Melanoplus differentialis* and *Schistocerca americana*, with the same results. In the case of *M. differentialis* no growth of *Fusarium vasinfectum* was obtained in cultures after approximately 45 minutes from the time the last spore-covered leaves had been eaten. For *S. americana* the period was 42 minutes (at 26.5° C.).

From these results it appears that the passage of *Fusarium vasinfectum* through the alimentary tracts of the insects used in these tests was relatively rapid.

This conclusion is substantiated by the results of cultures of pupae and adults of *Alabama argillacea*, the larvae of which had been fed upon spore-contaminated leaves for several days prior to pupation. In cultures of 49 of these pupae that had spun up in heavily inoculated leaves, only 2 yielded growth of *F. vasinfectum*. In cultures of 20 adults that were collected as they emerged from their pupal cases, no wilt fungus was obtained. There is little likelihood that the fungus is retained within *A. argillacea* in a viable state during pupation and formation of the imago.

RECOVERY OF FUSARIUM VASINFECTUM FROM BOLL-FEEDING INSECTS

Boll-feeding insects are usually numerous in cotton fields. Two types were worked with, the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis obsoleta* Fab.). Only entire immature stages and adult boll weevils collected from within bolls on badly wilted cotton plants were cultured. Three out of ninety-five larvae, or 3.2 percent, 1 out of 30 pupae, or 3.3 percent, and 1 out of 61 adults, or 1.6 percent, yielded pure-culture growth of *Fusarium vasinfectum*. The results of these tests with the boll weevil are not considered as conclusive of the passage of viable fungus through the alimentary tract and need further verification. Fecal pellets from *H. obsoleta* larvae that had fed upon spore-covered involucral bracts and small bolls were cultured after external sterilization. Pure growth of *F. vasinfectum* was obtained from 60 percent of these.

DISSEMINATION OF FUSARIUM VASINFECTUM THROUGH EXTERNALLY ADHERING SPORES

Numerous tests were made to determine the ability of insects to spread the cotton-wilt fungus on their appendages. After being exposed to cotton stems bearing an abundance of sporodochia, such insects as *Anthonomus grandis* Boh., *Olla abdominalis* Say, *Jadera haematoloma* H. S., *Zelus cervicalis* Stål, *Nezara viridula* L., *Euschistus servus* Say, and others were found to start cultures of *Fusarium vasinfectum* where their feet were permitted to touch the surface of acidified agar in Petri dishes. Cultures of the appendages of insects captured in badly wilted cotton fields showed that many normally transport viable fungus in this manner.

DISCUSSION

This paper has indicated the possibility of insects spreading *Fusarium vasinfectum* through their fecal pellets. Such pellets may carry the fungus as bits of hyphae or spores, which are unaffected by brief surface sterilization with an aqueous solution containing, by weight, 0.05 percent mercuric chloride and 35 percent ethyl alcohol.

To check the reliability of the results, cultures were made of fecal pellets dissected from the recta under aseptic conditions, and typical growth of *Fusarium vasinfectum* was obtained from the following insects: *Schistocerca americana*, *Melanoplus differentialis*, *M. mexi-*

canus, Chortophaga viridifasciata var. *australior, Encoptolophus texensis, Trimerotropis citrina,* and *Spharagemon cristatum.*

That the results obtained are largely accurate is further indicated by the negative results obtained with certain soil-inhabiting insects. If the methods had allowed contaminations sufficient to account for the large percentage of positive results obtained in many experiments, one might expect that those tests which were habitually negative would also have been subject to the same degree of contamination and that positive results would have been intermixed with negative ones. The same logic applies to the test in which cultures were made of individual pellets, as these were defecated from larvae of *Alabama argillacea* and grasshoppers that had been fed successively upon wilt-free food, spore-covered food, and wilt-free food. If the positive results had originated in external contaminations, there would have been no such abrupt change in the positive or negative character of cultures.

Another test may be cited as an indication of the accuracy of the results. Living *Alabama argillacea* larvae, containing wilt fungus internally, were submerged in the sterilization fluid for 30 seconds. This exposure was found to be not immediately fatal. The larvae were then transferred with sterile forceps to sterile acid-agar plates, where they were left until they had defecated one or more fecal pellets. The insects were then removed and the plate was left for fungus development. All of a number of tests resulted positively. In every instance the growth of *Fusarium vasinfectum* originated with fecal pellets. That external sterilization was efficient is indicated by the fact that not a single growth of the wilt fungus resulted from contact of larvae with agar surfaces during their wanderings inside the Petri dishes.

The point of appearance of *Fusarium vasinfectum* in cultures also merits consideration. When entire insects were externally sterilized and cultured, growths of the wilt fungus most commonly made their appearance near the anus, and more rarely on appendages and external body parts.

Passage of the cotton-wilt fungus through the alimentary tract of the insects studied appears to have been relatively rapid. Swiftly flying insects could undoubtedly spread *Fusarium vasinfectum* from field to field through their fecal pellets, although the supply of fungus would be exhausted before they could cover great distances. Those insects that do carry wilt fungus internally are probably of importance in field-to-field spread, and in intensifying the infective element within a field. Each infective fecal pellet deposited is an additional source of fungus that may ultimately attack cotton plants. The fecal pellet itself provides sufficient nutrient material to enable the fungus to grow. The possibilities with respect to insects that serve as vehicles for externally adhering spores are almost unlimited.

It is worthy of note that those insects whose alimentary fluids had no immediate lethal effect on *Fusarium vasinfectum* are all phytophagous. Most of the insects that destroyed wilt fungus by eating it, particularly the Collembola, feed normally upon decaying organic matter and fungi. Indications are that the association between insects and the wilt fungus is entirely a mechanical one.

SUMMARY

The following cotton insects were caged and fed on roots, stems and leaves, or bolls of cotton plants infected with typical fusarium wilt: *Melanoplus femur-rubrum*, *M. differentialis*, *Schistocerca americana*, *S. obscura*, and other less abundant grasshoppers; the larval stages of *Alabama argillacea*, *Laphygma frugiperda*, and *Prodenia ornithogalli*; a number of species of white grubs; and the larval stage of *Ataxia crypta*. Viable *F. vasinfectum* was recovered from fecal pellets or entire insects cultured on potato-dextrose agar in Petri dishes. The wilt fungus could not be recovered from, and was apparently destroyed while passing through, the alimentary tract of wireworms, Collembola, and Japygidae.

Viable *Fusarium vasinfectum* was recovered from cultures of the entire insects or of fecal pellets dissected out from the recta of numerous species collected in badly wilted cotton fields in Texas. The following insects were found to act as natural carriers of the cotton wilt fungus: *Melanoplus differentialis*, *M. mexicanus*, *M. femur-rubrum*, *Encoptolophus texensis*, *Spharagemon cristatum*, *Tomonotus aztecus*, *Chortophaga viridifasciata* var. *australior*, *Schistocerca americana*, *S. obscura*, *Trimerotropis citrina*, and *Dissosteira carolina*. It still remains to be proved whether the boll weevil can act as a carrier of fusarium wilt of cotton.

Cotton seedlings were successfully inoculated with strains of *Fusarium* isolated from the alimentary tract of insects and with a pure culture of *F. vasinfectum* from infected cotton.

Fusarium vasinfectum has survived for 15 months in fecal pellets from grasshoppers fed on wilt-infected cotton stems and kept dry in the laboratory.

It is suggested that these results may help to explain the occasional finding of infected plants in areas where the disease does not ordinarily occur.

RELATION OF THE CAROTENE CONTENT OF CERTAIN FEED MATERIALS TO THEIR VITAMIN A POTENCY [1]

By G. S. Fraps, *chief, Division of Chemistry*, Ray Treichler, *assistant chemist*, and A. R. Kemmerer, *assistant chemist, Texas Agricultural Experiment Station*

INTRODUCTION

It is now known that alpha-, beta-, and gamma-carotene, crypto-xanthin, and some other yellow pigments of plants can be utilized for the same purposes as vitamin A (*5, 7*).[2]

Carotene can be determined in a few hours, whereas the biological determination of vitamin A potency requires several weeks. Variations in carotene content can be easily ascertained chemically. The determination of carotene therefore offers a rapid method for estimating the vitamin A potency of many feeds, provided the relation between the carotene content of a particular feed and its biological value is known. Hence the relation between the carotene content of feeds and their vitamin A potency is of considerable practical as well as scientific importance. In connection with vitamin A investigations at the Texas Agricultural Experiment Station, it was necessary to determine the vitamin A potency of a number of feeds. Advantage was taken of the material available to determine the carotene content also and to secure information regarding the ratio mentioned.

EXPERIMENTAL PROCEDURE

The feeds were assayed biologically for vitamin A potency by means of the modified Sherman-Munsell method employed in this laboratory (*2*). The results were expressed both as Sherman-Munsell units and as international units. The international units were calculated by multiplying the number of Sherman-Munsell units by 1.2. In this laboratory, 1.2 international units have been found to be equal to 1 Sherman-Munsell unit.

Some of the feeds tested were ground and stored at room temperature during the fall, winter, and spring months; others were stored in an electric refrigerator. The carotene was determined at approximately the same time that the biological assays were made in order to minimize any error that might result from loss of carotene during storage.

The carotene in the various samples was determined by a modification of the method worked out by Guilbert (*3*) consisting in brief as follows: 2 to 5 grams of the feed sample was refluxed with aldehyde-free alcoholic potash for 30 minutes. The carotinoids were then extracted from the resulting solution by diluting with water and shaking with ethyl ether. The ether was then washed with distilled water and evaporated off under reduced pressure. The residue remaining was taken up in light petroleum ether and the xanthophyll removed by shaking with 85 and 90 percent methanol. The carotene

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was determined by comparing the petroleum ether solution in a colorimeter with a 0.1 percent solution of potassium dichromate which had been standardized against 0.001 percent carotene solution. All the results obtained were expressed as micrograms of carotene per gram of feed. No attempt was made to differentiate between the several active pigments.

RESULTS

A total of 15 samples of feeds are reported for carotene and vitamin A potency. There were seven samples of alfalfa meal, five samples of peanut hay, two samples of yellow corn, and one sample of Johnson grass hay.

The carotene of the alfalfa varied from 7.3 to 63.5 micrograms per gram, while the vitamin A potency varied from 13 to 77 Sherman-Munsell units (15.6 to 92.4 international units) per gram. The data are given in table 1. In the seven samples of alfalfa products, 1 microgram of carotene had a vitamin A potency of from 1.0 to 1.8 Sherman-Munsell units (1.2 to 2.1 international units) with an average of 1.4 Sherman-Munsell units (1.6 international units). The international units per microgram of carotene were secured by dividing the international units per gram of feed by the microgram of carotene per gram of feed. If the Sherman-Munsell units per microgram of carotene are multiplied by 1.2, slightly different results are secured in some cases because of the rounding off of the fractions before the Sherman-Munsell units per microgram of carotene were tabulated.

TABLE 1.—*Relation of carotene in feeds to their vitamin A potency*

Laboratory no.	Kind of feed	Vitamin A potency per gram feed		Micrograms of carotene per gram of feed	Units of vitamin A potency per microgram of carotene	
		Sherman-Munsell units	International units		Sherman-Munsell units	International units
41727	Alfalfa meal	60	72.0	48.1	1.2	1.5
41745	Alfalfa leaf meal	67	80.4	53.1	1.3	1.5
41952	Alfalfa hay	13	15.6	7.3	1.8	2.1
41967	Dehydrated alfalfa leaf meal	77	92.4	59.4	1.3	1.6
42622	___do	75	90.0	63.5	1.2	1.4
41728	Alfalfa leaf meal	25	30.0	25.0	1.0	1.2
41277	Dehydrated alfalfa leaf meal	60	72.0	33.6	1.8	2.1
	Mean				1.4	1.6
41724	Peanut hay	8	9.6	5.6	1.4	1.7
41726	___do	17	20.4	13.3	1.3	1.6
41732	___do	38	45.6	26.5	1.4	1.7
41733	___do	15	18.0	13.9	1.1	1.3
41734	___do	25	30.0	21.4	1.2	1.4
	Mean				1.3	1.5
41110	Yellow corn	2	2.4	1.7	1.2	1.4
41229	___do	3	3.6	2.6	1.2	1.4
	Mean				1.2	1.4
41951	Johnson grass hay	10	12.0	9.3	1.1	1.3

Peanut hay ranged in vitamin A potency from 5.6 to 26.5 micrograms of carotene per gram and from 1.1 to 1.4 Sherman-Munsell units (1.3 to 1.7 international units) per microgram of carotene with an average of 1.3 (1.5 international units) for the five samples.

The two samples of yellow corn had a value of 1.2 Sherman-Munsell units (1.4 international units) of vitamin A potency per microgram of carotene, while the sample of Johnson grass hay had a vitamin A potency of 1.1 Sherman-Munsell units (1.3 international units) per microgram of carotene.

Results from feeding rats purified carotene dissolved in oil show that 1.0 Sherman-Munsell unit is equivalent to 0.72 microgram of beta-carotene. This makes 1 microgram of beta-carotene equal to 1.4 Sherman-Munsell units (table 2). The same results were obtained by feeding the international standard. One Sherman-Munsell unit was found to equal 1.2 international units. Since 1 international unit is 0.6 microgram of beta-carotene, 1.2 international units are equal to 0.6 × 1.2 or 0.72 microgram of beta-carotene. Since the average value of 1 microgram of carotene in alfalfa products and in peanut hay was 1.4 and 1.3 Sherman-Munsell units, respectively, the biological value of carotene in the international standard, in the purified carotene, and in the alfalfa and peanut hay products are practically the same. The differences found are within the limits of experimental error. This result is similar to that of Morgan and Madsen (8), who found the value of the carotene in apricots to be nearly 100 percent of the expected value.

TABLE 2.—*Relation of carotene content to vitamin A potency when rats were fed various feeds and purified carotene in oil*

Material	Vitamin A potency per microgram of carotene	
	Sherman-Munsell units	International units
Alfalfa products	1.4	1.6
Peanut hay	1.3	1.5
Yellow corn	1.2	1.4
Johnson grass hay	1.1	1.3
International standard	1.4	1.7
Purified carotene in oil	1.4	1.7

Practically all the carotene of alfalfa hay and peanut hay should be beta-carotene, as has already been reported for alfalfa hay by Hartman, Kane, and Shinn (4). Mackinney (6) found the major carotene fraction of leaves to consist of beta-carotene; grass contained only beta-carotene with no detectable amounts of alpha-carotene present.

The average number of Sherman-Munsell units per microgram of carotene was less for two samples of yellow corn than it was in alfalfa or peanut hay. This may be accounted for by the fact that the actual pigments in yellow corn consist in large part of cryptoxanthin (40 to 70 percent, according to the writers' results), which has a lower vitamin-A potency than beta-carotene. However, there may be appreciable errors in the results with yellow corn because too few samples were used. More data are being obtained for this feed. The carotene in one sample of Johnson-grass hay also had less vitamin A potency than the average for carotene in peanut or alfalfa hay.

It is possible that at higher levels of feed there may be a poorer utilization of carotene. Ahmad (1) found a variation in the utilization of ingested carotene due to imperfect absorption from the gut. This

and other factors such as differences in digestibility due to the nature of the plant tissue and differences in the kind of carotene found may cause variations in the utilization of plant carotenes at higher levels. This work is being continued.

SUMMARY

Seven samples of alfalfa products containing from 7.3 to 63.5 micrograms of carotene had a vitamin A potency of from 13 to 77 Sherman-Munsell units (15.6 to 92.4 international units) per gram, with an average value of 1.4 units (1.6 international units) per microgram of carotene. Five samples of peanut hay ranging from 5.6 to 26.5 micrograms of carotene per gram had an average value of 1.3 Sherman-Munsell units (1.5 international units) per microgram of carotene.

Two samples of yellow corn containing 1.7 and 2.6 micrograms of carotene per gram had an average value of 1.2 Sherman-Munsell units (1.4 international units) per microgram of carotene, while one sample of Johnson grass hay containing 9.3 micrograms of carotene per gram had a vitamin A potency of 1.1 Sherman-Munsell units (1.3 international units) per microgram of carotene.

One microgram of carotene in the international standard had a value of 1.4 Sherman-Munsell units, and 1 microgram of purified carotene had the same value. Carotene in alfalfa products and peanut hay had practically the same vitamin-A potency, expressed in Sherman-Munsell units, as the carotene in the international standard and in purified carotene dissolved in oil.

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INFLUENCE OF ENVIRONMENT DURING MATURATION ON THE DISEASE REACTION AND YIELD OF WHEAT AND BARLEY[1]

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INTRODUCTION

The influence of size of seed on development and yield of plants has been studied by numerous workers. Little attention has been given however, to the influence, on the subsequent crop, of other characteristics of the seed determined by the environment while the seed was attached to the mother plant, or to the effect of seed characteristics on other aspects of the crop besides yield. The experiments reported herein were undertaken to study reaction to seedling blight caused by *Gibberella saubinetii* (Mont.) Sacc.; reaction to bunt caused by *Tilletia levis* Kühn; and yield from seed produced in different environments. For this purpose seed of varieties of wheat and barley, produced at a number of places in the United States and Canada, was tested for reaction to seedling blight in the greenhouse at Madison, Wis., and for reaction to bunt and for yield in field trials at Moccasin, Mont., Madison, Wis., and Aberdeen, Idaho. Although the primary purpose of the experiments was to determine the effect of different environments on disease reaction and yield of seed lots of the same variety, data were also obtained on the relative resistance of the varieties to seedling blight.

REVIEW OF LITERATURE

Some original data and a review of the literature dealing with the subject of physiologic predetermination have been presented by Kidd and West (8, 9).[3] They conclude, largely on the basis of the literature, that (1) larger seeds give rise to more vigorous plants and a better yield, and that (2) the environment under which seed is produced may have a marked influence on the development of plants of the next generation. Their evidence consists chiefly of the effects of size of seed.

Seed of certain spring wheat varieties produced in central Illinois was found by Dickson et al. (5) to be more susceptible to *Gibberella saubinetii* than seed of the same varieties produced at Madison, Wis. Similar differences were found with late-maturing types of corn,

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except that seed produced at Madison was the more susceptible. These workers report small differences in the composition of the endosperm but marked differences in the composition of the embryo. Hoppe et al. (7) also found that the environment during the period of growth and maturation of the mother plant greatly affects the susceptibility of corn seedlings to *G. saubinetii*. They harvested self-pollinated corn at intervals and found that the degree of maturity of the seed influenced the disease reaction and yield of the subsequent crop.

᠊Many workers have studied the relation of size of seed to germinatioñ growth of the plant, and yield. In all the experiments, however, seed of the different sizes has been obtained by separations made with screens or a fanning mill, and not by producing the seed under different environmental conditions. Kiesselbach and Helm (10) have presented a comprehensive review of the literature dealing with this subject, and it seems unnecessary to present another review at this time. They also conducted an elaborate series of experiments, and their conclusions summarize the information on the effect of size of seed on yield, as follows (10, p. 69):

(1) When space-planted to permit maximum development, a higher individual plant yield is obtained from large than from small seeds.   *   *   *
(2) When planted in equal numbers at a rate optimum for large seed, a lower yield is obtained from the small than from the large seed.   *   *   *
(3) When planted in equal weights, at a rate optimum for the large seed, all three grades—large, small, and unselected—yield equally.

Arny and Garber (1) made a critical study of the relation of seed size to rate of growth of spaced plants and yield in wheat. They found a positive correlation between size of seed and size of plants. The maximum difference in size of plants occurred at 6 weeks of age, with a tapering off of the difference as the plants approached maturity. They also found a positive correlation between size of seed and yield, the relation being more pronounced on poor than on fertile soil.

Kucheryaeva (11) found that immature seed, harvested when the kernels weighed 10 g per 1,000, yielded the same in a favorable year as mature seed from the same crop that weighed 29 g per 1,000 kernels. Differences in favor of the larger seed were obtained, however, in dry, unfavorable years.

MATERIAL AND METHODS

Four varieties of spring wheat (*Triticum aestivum* L., syn. *T. vulgare* Vill.) and three of barley (*Hordeum vulgare* L.) were chosen for the present study and were grown at 10 different locations in the United States and Canada in 1929 and at 12 locations in 1930. The wheat varieties were Marquis (C. I.[4] 3641), Hard Federation (C. I. 4733), Peliss selection 89, and Illinois No. 1. The barley varieties were Trebi (C. I. 936), Hannchen selection 1 (C. I. 5462), and Oderbrucker (Wisconsin Pedigree No. 5) (C. I. 1272). All but Illinois No. 1 wheat had been carefully selected and the seed used was reasonably pure. The Illinois No. 1 was an unselected type and was found to be mixed. Illinois No. 1A, a mass-selected strain, was substituted for it in 1930.

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[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

The 10 locations at which seed was produced in 1929 and the 12 at which it was produced in 1930, together with yield of the crop, weight of 1,000 kernels, and protein content of the grain from each, are given in tables 1 and 2. Graphs showing the weight per 1,000 kernels and the protein content are presented later (see figs. 1, 2, 4, 5, and 6) in connection with other data. Seed was sent to each station for sowing each year to insure use of the same seed at all stations. Each variety was grown in three nursery plots of three rows each, the center row only being harvested for yield. All seedings were made at the normal rate and date at each station.

TABLE 1.—*Yield and protein content of the grain and weight of 1,000 kernels of 4 varieties of wheat and 3 of barley, grown at 10 experiment stations in the United States and Canada in 1929*

WHEAT VARIETIES

Station where grown	Marquis			Illinois No. 1			Hard Federation			Peliss selection 89		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bu.	*Pct.*	*G*	*Bu.*	*Pct.*	*G*	*Bu.*	*Pct.*	*G*	*Bu.*	*Pct.*	*G*
Aberdeen, Idaho	41.5	11.4	35.1	38.0	11.8	34.0	42.8	11.1	39.8	43.3	11.0	61.1
Bozeman, Mont	37.4	16.7	34.8	31.0	16.3	30.5	45.6	13.6	35.6	60.9	14.6	60.6
Felt, Idaho	17.2	15.6	32.8	21.3	14.0	28.6	24.1	16.5	35.6	26.8	11.1	55.2
Hays, Kans	5.1	17.6	16.6	6.7	13.4	17.4	10.3	16.7	18.2	12.8	18.6	27.0
Indian Head, Saskatche- wan	34.1	15.3	29.0	31.3	17.1	28.5	29.6	14.4	34.8	50.3	15.1	52.4
Madison, Wis	2.9	13.9	14.8	2.7	12.9	15.8	1.7	12.8	15.2	.3		30.2
Mandan, N. Dak	5.7	15.4	19.2	4.9	14.2	20.0	4.9	14.8	23.0	7.1	15.8	36.3
Moccasin, Mont	8.2	18.2	18.0	9.7	19.1	18.4	7.6	16.9	24.2	10.8	17.5	29.7
Moro, Oreg	13.2	14.9	20.2	11.7	15.2	24.8	18.3	13.6	28.9	15.9	14.6	37.0
North Platte, Nebr	17.8	16.8	19.4	14.1	16.1	19.0	15.2	14.5	18.8	21.6	16.1	39.1

BARLEY VARIETIES

	Hannchen selection 1			Trebi			Oderbrucker		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bushels	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*
Aberdeen, Idaho	63.4	12.1	42.8	67.1	11.8	48.1	28.6	13.2	37.2
Bozeman, Mont	63.4	13.3	38.3	68.5	11.9	44.9	42.9	13.3	37.2
Felt, Idaho	37.3	14.3	37.7	30.0	12.1	44.2	11.5	14.2	33.7
Hays, Kans	23.6	15.5	17.2	35.1	15.2	22.6	16.5	14.6	16.3
Indian Head, Saskatchewan	50.4	13.3	31.2	56.0	12.2	34.7	33.3	13.6	26.0
Madison, Wis	19.3	12.4	29.4	8.9	12.3	36.5	9.4	11.4	26.1
Mandan, N. Dak	6.7	13.3	26.3	5.7	13.0	30.6	3.2	14.0	25.7
Moccasin, Mont	15.3	17.2	23.2	17.1	12.1	26.2	2.0	17.7	19.5
Moro, Oreg	24.1	12.3	24.9	23.9	12.8	27.1	4.4	13.5	20.2
North Platte, Nebr	31.3	13.7	19.2	30.0	13.4	26.5	18.0	14.9	20.3

TABLE 2.—*Yield and protein content of the grain and weight of 1,000 kernels of 4 varieties of wheat and 3 of barley, grown at 12 experiment stations in the United States and Canada in 1930*

WHEAT VARIETIES

Station where grown	Marquis			Illinois No. 1A			Hard Federation			Peliss selection 89		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bu.	*Pct.*	*G.*	*Bu.*	*Pct.*	*G.*	*Bu.*	*Pct.*	*G.*	*Bu.*	*Pct.*	*G.*
Aberdeen, Idaho	62.0	12.2	35.3	64.9	12.8	32.7	60.8	12.0	40.3	65.6	11.3	58.3
Arlington, Va	2.3	12.9	22.0	4.5	13.9	22.8	2.6	12.9	16.5	3.5	13.9	30.3
Bozeman, Mont	65.4	14.1	37.3	61.9	15.0	32.8	44.9	13.6	39.2	81.5	13.0	56.0
Felt, Idaho	17.0	14.8	27.4	14.7	16.2	27.1	9.2	15.8	31.1	20.7	14.4	47.1
Hays, Kans	6.3	15.6	13.4	5.3	15.5	13.1	5.6	14.9	15.0	8.4	15.7	27.2
Indian Head, Saskatchewan	39.8	14.2	27.1	40.2	14.0	23.8	36.1	10.8	27.1	69.0	15.4	51.2
Madison, Wis	16.1	12.3	15.4	15.4	11.8	13.3	8.5	11.3	10.4	22.5	13.7	36.8
Mandan, N. Dak	15.7	17.0	22.8	16.9	17.7	23.6	13.3	14.5	20.0	20.9	16.4	39.8
Moccasin, Mont	13.8	16.1	22.3	14.9	17.6	22.0	14.0	15.2	32.4	17.8	16.1	42.5
Moro, Oreg	10.1	16.3	19.7	8.7	16.3	21.4	11.3	14.5	23.2	10.5	16.4	29.2
North Platte, Nebr	21.3	15.3	17.0	21.1	14.4	16.6	15.8	12.2	17.5	22.5	15.6	28.5
Pendleton, Oreg	27.2	9.3	31.9	32.6	10.0	27.8	35.2	9.6	35.9	27.3	9.5	45.0

BARLEY VARIETIES

	Hannchen selection 1			Trebi			Oderbrucker		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bushels	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*
Aberdeen, Idaho	97.0	9.9	39.8	96.4	9.6	43.8	50.5	10.7	35.5
Arlington, Va	12.6	11.0	32.1	11.8	10.5	35.5	4.8	12.7	29.1
Bozeman, Mont	87.2	12.1	38.6	86.3	11.2	43.8	76.6	12.5	36.1
Felt, Idaho	29.4	16.3	31.1	21.5	15.2	34.0	4.8	15.6	29.0
Hays, Kans	11.4	13.2	23.2	47.8	11.7	35.1	9.8	14.4	17.0
Indian Head, Saskatchewan	87.6	14.2	35.3	92.6	12.7	42.7	79.6	13.5	33.7
Madison, Wis	33.0	11.1	32.7	53.2	11.7	40.3	28.9	13.0	29.3
Mandan, N. Dak	29.8	15.3	30.7	19.3	14.6	27.6	16.1	15.1	25.1
Moccasin, Mont	19.6	16.4	25.3	28.2	14.5	29.8	14.8	16.5	25.3
Moro, Oreg	13.1	16.5	27.4	9.8	16.2	22.8	3.5	16.7	22.0
North Platte, Nebr	37.3	12.7	24.2	51.4	12.1	31.8	38.5	12.7	25.5
Pendleton, Oreg	53.5	7.5	39.4	65.1	7.3	42.5	25.6	8.5	34.5

As would be expected, seed produced at the several stations varied greatly in protein content and weight of 1,000 kernels. Kernels of all varieties produced at Aberdeen, Bozeman, Pendleton, and Felt were large, in some cases weighing more than twice as much as those produced elsewhere, as, for example, at Hays, Moccasin, North Platte, and Madison. Seed of most of the varieties from Moccasin, Hays, and Felt was high in protein content, whereas that from Aberdeen, Madison, and Pendleton was low.

In order to obtain seed that differed in size owing to the stage at which it was harvested, a large number of heads in border rows of the Marquis check plots at Moccasin, Mont., were tagged when anthers protruded from florets near the center of the heads. In 1929, the heads were tagged on July 16 and 17 and harvested on July 27 and August 3, 9, and 14, on which dates the kernels were in early

milk, soft-dough, hard-dough, and mature stages, respectively. In 1930, heads were tagged on July 8 and harvested daily from July 12 to 19 and at longer intervals to maturity. The heads were cut from the stems and spread on a screen in the sun to dry rapidly. The weight of 1,000 kernels for each of these samples is given in table 7, in connection with the data on resistance to seedling blight.

The tests for reaction to seedling blight caused by *Gibberella saubinetii* were conducted in soil in the greenhouse at Madison. Considerable difficulty was encountered in perfecting a technique that would give a differential infection. Conidial suspensions used as inoculum proved unsatisfactory. On the other hand, mycelial cultures grown for about 10 days on a medium of 1 part corn meal, 1 part sand, and 1½ parts water, which had been steamed for about 30 minutes and sterilized in an autoclave, produced satisfactory infections. The culture was mixed with sandy soil in the proportion of 150 parts soil to 1 part culture, and 150 cc of the mixture was sown with the seed in each 18-inch row. Strain no. 1 [5] of the organism was used in testing the seed lots produced in 1929 and strain no. 73C in testing those produced in 1930. It was not considered necessary to use the same strain each year, as it was desired only to determine whether differences occur in reaction to blight in seedlings of the same variety of wheat or barley from seed produced under different environmental conditions. The tests were conducted in January and February 1930 for the seed lots produced in 1929, and in January and February 1931 for those produced in 1930. Each seed lot tested for reaction to seedling blight was also grown without inoculation to check viability and natural infection of seedling blight organisms. Except as noted otherwise in the table headings, the greenhouses were kept at temperatures averaging about 19° C.

In taking disease notes on the seedlings, the plants were divided into six classes, as follows: (1) Healthy; (2) having lesions on the seminal roots; (3) having lesions on coleoptile, but not stunted; (4) having lesions on coleoptile or deep-seated lesions, and stunted; (5) blighted after emergence; and (6) blighted before emergence (determined by subtracting the number of plants emerging from the number of seeds planted). Cracked kernels were not used, and viability was high in most of the controls. In order to convert the data to a single figure or resistance index for each seed lot, the following values were assigned to each class: Class 1, 1.0; class 2, 0.8; class 3, 0.7; class 4, 0.3; class 5, 0.2; and class 6, 0. These values, on the basis of 100 seeds sown, give a resistance index of 100 for a seed lot that produced 100 healthy plants and 0 for a seed lot that produced no plants.

Tests of wheat for reaction to bunt were conducted in the field at Moccasin and Madison. The seed was shaken in an envelope with an excess of spores from bunted heads of wheat collected at Moccasin. Infection was based on the percentage of heads showing any smut balls.

Yield tests of seed produced at the different locations were conducted in nursery rows at Madison and Moccasin in 1930 and 1931, and at Aberdeen in 1931. The test at Aberdeen was grown under irrigation.

[5] Strain numbers refer to numbers of the collections of *Gibberella saubinetii* of the Department of Plant Pathology, University of Wisconsin.

EXPERIMENTAL RESULTS

REACTION TO SEEDLING BLIGHT

RESULTS WITH SEED PRODUCED IN 1929

All of the seed lots produced at the several stations in 1929 were tested for reaction to seedling blight caused by *Gibberella saubinetii* in two separate tests in the greenhouse at Madison. In each test duplicate rows of 100 seeds each were inoculated; one row was not inoculated in order to determine the viability and natural infection the seed. In the first test both wheat and barley were sown January 1, 1930, and notes were taken January 20 to 24, when most of the plants were in the third-leaf stage. In the second test the barley was sown February 1 and the wheat February 8, and the notes were taken when most of the plants were in the fourth- or fifth-leaf stage.

The indices of resistance for both tests are given for wheat in table 3 and for barley in table 4. The resistance indices, the weight of 1,000 kernels, and the protein content of the seed sown are shown in figure 1 for the wheat varieties Marquis, Illinois No. 1, Peliss selection 89, and Hard Federation, and in figure 2 for the three barley varieties. In the second test the infection on inoculated wheat was so heavy that resistance indices of seed from several stations approached 0 and differences were obscured; therefore, in figure 1 the data from the first test were used instead of the averages of the two tests.

TABLE 3.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 4 varieties of wheat, from seed produced at 10 experiment stations in 1929, when grown in the greenhouse at Madison, Wis.*

INOCULATED WITH GIBBERELLA SAUBINETII

Station where seed was produced	Resistance index												Average of 4 varieties
	Illinois No. 1			Marquis			Hard Federation			Peliss selection 89			
	First test	Second test	Average	First test	Second test	Average	First test	Second test	Average	First test	Second test	Average	
Madison, Wis	82.2	43.6	62.9	73.4	32.2	52.8	47.9	28.3	38.1	49.5	[1] 4.9	27.2	45.3
North Platte, Nebr	62.2	18.2	40.2	57.5	17.8	37.7	55.4	20.8	38.1	60.8	10.8	35.8	38.0
Indian Head, Saskatchewan	70.9	25.0	48.0	49.5	3.9	26.7	33.2	1.1	17.2	42.2	4.6	23.4	28.8
Aberdeen, Idaho	40.1	4.3	22.2	52.6	18.0	35.3	24.4	3.2	13.8	40.3	6.8	23.6	23.7
Bozeman, Mont	44.3	9.7	27.0	35.5	2.8	19.2	25.7	1.8	13.8	32.0	2.1	17.1	19.3
Moro, Oreg	43.3	2.5	22.9	42.1	1.5	21.8	27.6	.3	14.0	23.7	.8	12.3	17.8
Hays, Kans	29.0	.4	14.7	28.7	1.3	15.0	46.4	14.3	30.4	13.6	0	6.8	16.7
Moccasin, Mont	40.5	2.1	21.3	31.6	.3	16.0	25.5	.6	13.1	25.1	1.4	13.3	15.9
Felt, Idaho	41.7	1.7	21.7	41.0	1.2	21.1	25.7	2.0	13.9	10.6	.3	5.5	15.6
Mandan, N. Dak	35.1	1.1	18.1	31.9	.3	16.1	23.9	1.9	12.9	23.9	0	12.0	14.8
Average	48.9	10.9	29.9	44.4	7.9	26.2	33.6	7.4	20.5	32.2	3.2	17.7	23.6

CONTROL (NOT INOCULATED)

Station where seed was produced													
Madison, Wis	97.4	95.5	96.5	95.8	92.4	94.1	92.5	94.1	93.3	86.0			92.5
North Platte, Nebr	87.0	97.4	92.2	94.7	94.1	94.4	97.3	97.7	97.5	80.6	87.2	83.9	92.0
Indian Head, Saskatchewan	99.0	97.3	98.2	100.0	98.4	99.2	97.2	98.2	97.7	98.2	98.2	98.2	98.3
Aberdeen, Idaho	91.4	94.3	92.9	97.2	96.5	96.9	92.0	94.5	93.3	90.2	89.9	90.1	93.3
Bozeman, Mont	89.7	87.1	88.4	93.2	89.7	91.5	81.3	80.8	81.1	92.6	93.7	93.2	88.6
Moro, Oreg	92.3	92.7	92.5	94.4	88.3	91.4	83.3	85.3	84.3	91.4	80.8	86.1	88.6
Hays, Kans	92.8	89.9	91.4	95.5	95.4	95.5	89.1	88.8	89.0	76.5	73.5	75.0	87.7
Moccasin, Mont	92.8	91.7	92.3	91.8	96.4	94.1	92.6	85.6	89.1	83.5	86.6	85.1	90.2
Felt, Idaho	89.8	88.5	89.2	84.6	88.1	86.4	93.1	94.0	93.6	54.8	54.5	54.7	81.0
Mandan, N. Dak	91.1	91.5	91.3	87.9	81.3	84.6	84.0	95.4	89.7	77.4	82.2	79.8	86.4
Average	92.3	92.6	92.5	93.5	92.1	92.8	90.2	91.4	90.9	83.1	83.0	82.9	89.9

[1] Interpolated.

TABLE 4.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 3 varieties of barley, from seed produced at 10 experiment stations in 1929, when grown in the greenhouse at Madison, Wis.*

INOCULATED WITH GIBBERELLA SAUBINETII

Station where seed was produced	Resistance index									
	Hannchen selection 1			Trebi			Oderbrucker			Average of 3 varieties
	First test	Second test	Average	First test	Second test	Average	First test	Second test	Average	
Madison, Wis	76.8	66.6	71.7	81.5	77.8	79.7	84.3	78.7	81.5	77.6
Indian Head, Saskatchewan	70.1	58.2	64.2	67.0	66.0	66.5	67.4	57.2	62.3	64.3
North Platte, Nebr	67.3	54.7	61.0	64.9	67.0	66.0	66.2	58.3	62.3	63.1
Felt, Idaho	62.0	51.2	56.6	64.5	64.8	64.7	70.0	60.9	65.5	62.3
Bozeman, Mont	63.1	55.1	59.1	63.0	61.0	62.0	69.0	61.2	65.1	62.1
Aberdeen, Idaho	66.0	57.5	61.8	69.1	56.5	62.8	65.8	56.6	61.2	61.9
Moro, Oreg	59.1	58.5	58.8	57.0	61.5	59.3	59.8	58.5	59.2	59.1
Hays, Kans	60.4	56.9	58.7	60.1	56.1	58.1	58.5	56.1	57.3	58.0
Mandan, N. Dak	61.0	53.6	57.3	62.6	53.1	57.9	57.5	52.5	55.0	56.7
Moccasin, Mont	55.9	47.5	51.7	57.4	54.2	55.8	57.4	50.8	54.1	53.9
Average	64.2	56.0	60.1	64.7	61.8	63.3	65.6	59.1	62.4	61.9

CONTROL (NOT INOCULATED)

Station where seed was produced	Hannchen selection 1			Trebi			Oderbrucker			Average of 3 varieties
Madison, Wis	96.1	87.8	92.0	93.1	93.0	93.1	93.9	92.6	93.3	92.8
Indian Head, Saskatchewan	95.5	95.5	95.5	97.2	97.3	97.3	98.0	98.1	98.1	97.0
North Platte, Nebr	95.7	91.0	93.4	95.2	95.4	95.3	94.3	94.0	94.2	94.3
Felt, Idaho	91.1	79.5	85.3	94.2	89.3	91.8	97.8	95.8	96.8	91.3
Bozeman, Mont	94.8	92.1	93.5	96.7	95.0	95.9	95.3	98.9	97.1	95.5
Aberdeen, Idaho	93.2	87.6	90.4	93.9	95.7	94.8	92.8	93.4	93.1	92.8
Moro, Oreg	94.1	97.4	95.8	90.6	92.8	91.7	96.6	89.8	93.2	93.6
Hays, Kans	81.8	87.6	84.7	89.8	90.1	90.0	93.9	85.5	89.7	88.1
Mandan, N. Dak	88.2	91.3	89.8	89.4	85.6	87.5	93.8	91.6	92.7	90.0
Moccasin, Mont	93.9	91.8	92.9	90.3	91.4	90.9	89.8	94.0	91.9	91.9
Average	92.4	90.2	91.3	93.0	92.6	92.8	94.6	93.4	94.0	92.7

The average resistance index for the four wheat varieties from Madison, as shown in table 3, was 45.3; from North Platte, 38.0; and from Indian Head, 28.8; while those from Mandan, Felt, and Moccasin averaged only 14.8, 15.6, and 15.9, respectively. The average resistance indices of the seed lots from other stations were intermediate. The relative rank of the stations was very similar for the barley varieties. Low viability and natural infection of the seed do not account for the differences, since there were marked differences in reaction in the inoculated series when the rows not inoculated showed similar resistance indices.

The average resistance indices for the seed lots of the four wheat varieties from all stations were as follows: Illinois No. 1, 29.9; Marquis, 26.2; Hard Federation, 20.5; and Peliss selection 89, 17.7. There were no significant differences for the barley varieties.

The differences between seed lots of all varieties produced at different stations were much greater than the average differences between varieties produced at the same stations. Thus for the wheat varieties the greatest difference was between Mandan and Madison, with resistance indices of 14.8 and 45.3, respectively. Among varieties the greatest difference was between Peliss selection 89 and Illinois No. 1, the indices being respectively 17.7 and 29.9. It would appear, therefore, that the place where the seed was grown had more influence on infection than did the variety. This was especially true for the barley, in which the differences between varieties were negligible.
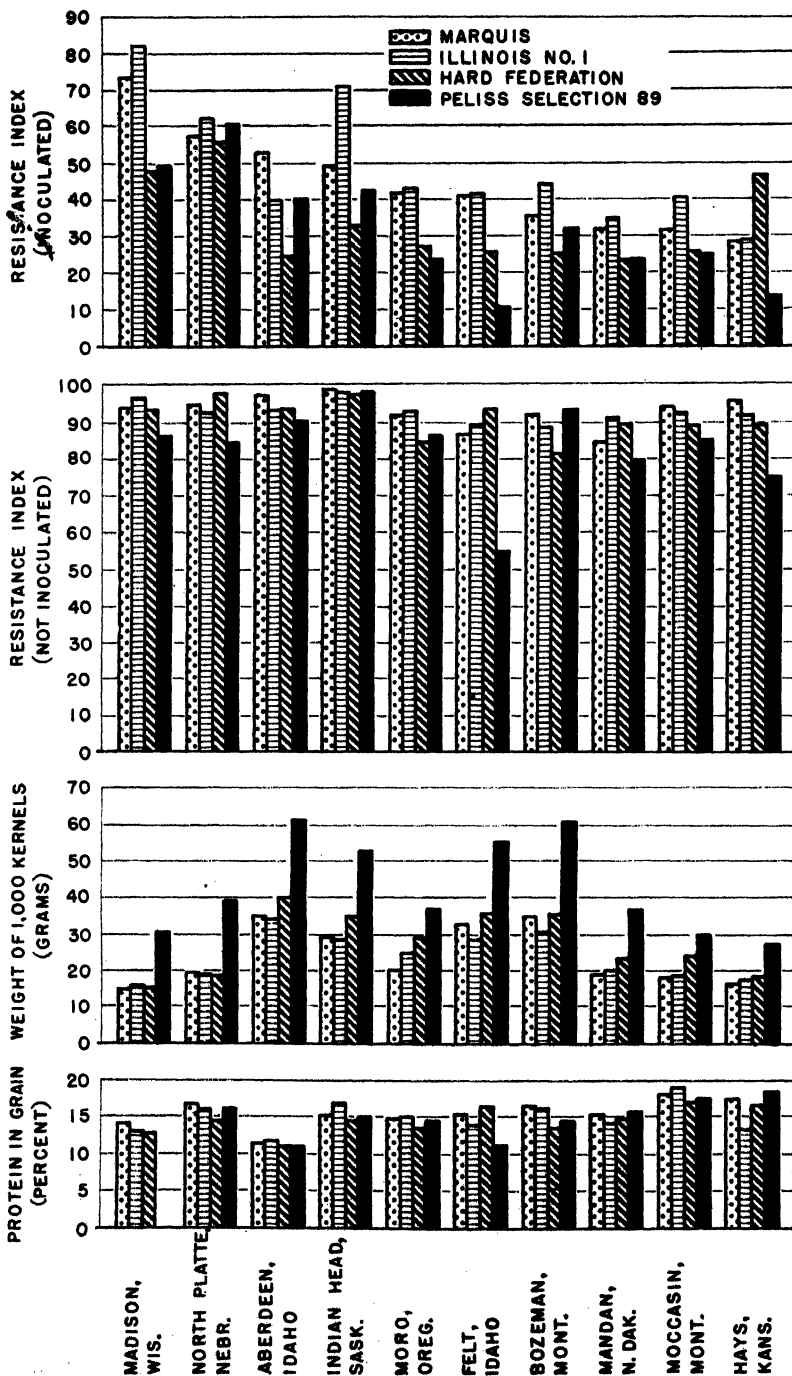
FIGURE 1.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of four varieties of wheat grown in the greenhouse at Madison, Wis., from seed produced at each of 10 experiment stations in 1929, and weight of 1,000 kernels and protein content of seed sown.
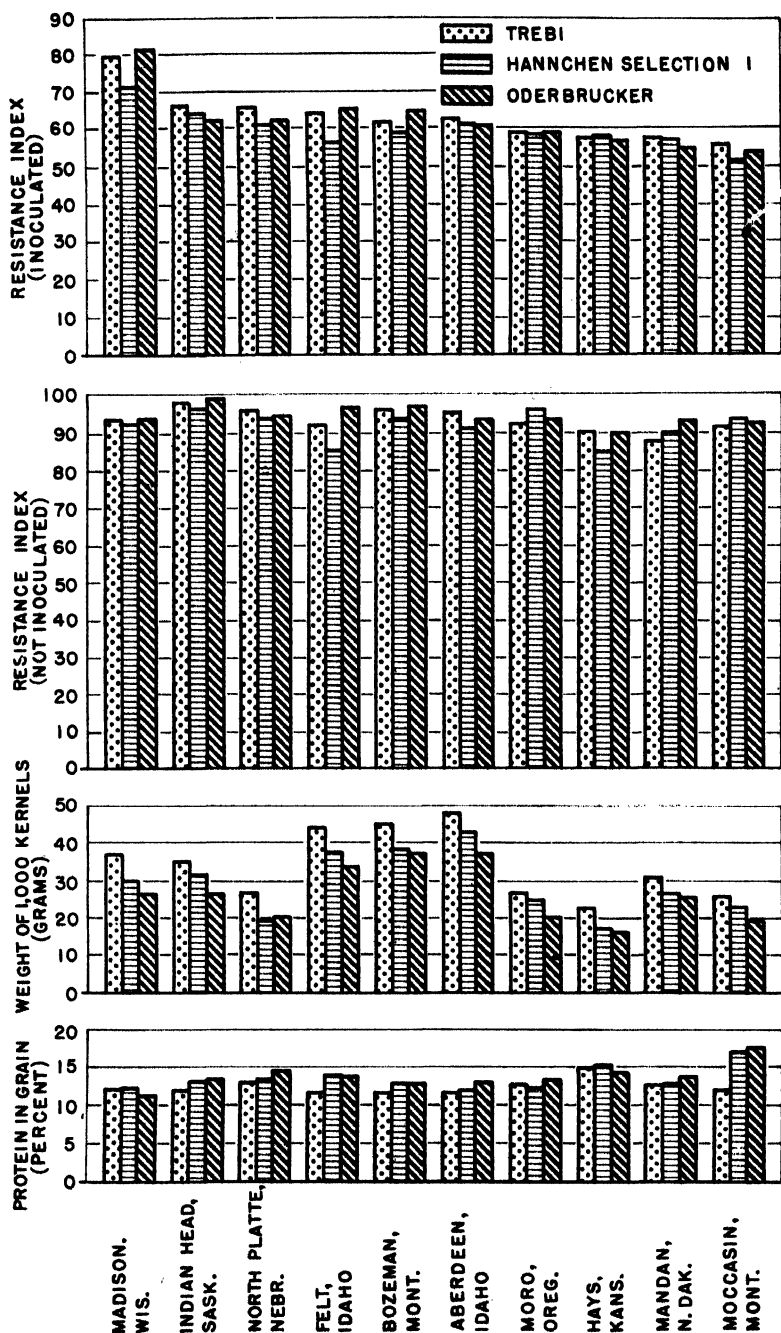
FIGURE 2.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of three varieties of barley grown in the greenhouse at Madison, Wis., from seed produced at each of 10 experiment stations in 1929, and weight of 1,000 kernels and protein content of seed sown.

Standard errors are not given for the data presented in tables 3 and 4, as the ranges in resistance indices are so great that use of a generalized error might be misleading. The significance of the data is indicated, however, by the variability of 11 checks of Marquis distributed throughout the first test. The standard deviation of these checks was 4.21, indicating definitely that the differences in resistance indices mentioned above are not due to random variation.

It will be noted from figure 1 that, excepting the seed lots from Aberdeen and Indian Head, the resistance indices for the inoculated seed of Marquis are only slightly lower than for Illinois No. 1. For seed from Aberdeen the resistance index of Marquis (52.6) was higher than for Illinois No. 1 (40.1), while for seed from Indian Head the resistance index for Marquis (49.5) was much lower than for Illinois No. 1 (70.9). Similar differences are apparent for Peliss selection 89 and Hard Federation. Peliss selection 89 shows a very low resistance index for seed produced at Felt, even when not inoculated. In this case, the grain of Peliss selection 89 was frosted before it was mature and the germination was injured; while the other varieties, being earlier, were ripe when frost occurred and were not injured. Hard Federation from Hays also produced a relatively high resistance index, as compared with the other varieties. This may have been due to its earliness, which allowed it to escape serious injury from heat and drought.

As shown in figure 2, all of the barley varieties were infected less than the wheat varieties and the differences between them were smaller. However, the Madison-grown seed of each variety had the highest resistance index and Moccasin-grown seed the lowest. Seed of all three varieties from the several stations ranked in about the same order in the inoculated seedings, with the exception of Hannchen selection 1 from Felt, which also had a low reading in the uninoculated control. These reactions would suggest, as might be expected, that varieties do not respond alike to all environments.

The weight of 1,000 kernels, the protein content, and the relation of these factors to reaction to seedling blight are discussed in a later section of this paper.

As an additional check on the reliability of the results presented in tables 3 and 4, seed of the 3 barley and 4 wheat varieties from 5 of the 10 experiment stations was grown at temperatures of 12°, 20°, and 26° C. Duplicate rows of 75 seeds each were inoculated with *Gibberella saubinetii* and 1 row of 75 seeds was not inoculated, at each temperature. Results from this test are presented in table 5. The resistance indices of Marquis and Illinois No. 1 grown in the two tests reported in table 3 and an average for those grown under the three temperatures reported in table 5 are shown in figure 3. The results for the inoculated seed lots of these two varieties of wheat from the five stations rank in practically the same order in each test (fig. 3). Results with the other varieties agree equally well. This similarity may be taken as verifying the conclusion that the major differences are not due to random errors.

TABLE 5.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 4 varieties of wheat and 3 varieties of barley, from seed produced at 5 experiment stations in 1929, when grown in the greenhouse at Madison, Wis., at 3 temperatures, in February 1930*

WHEAT VARIETIES

Variety and station where seed was produced	Grown at 26° C.			Grown at 20° C.			Grown at 12° C.			Resistance index average of 3 temperatures	
	Days to emergence	Resistance index		Days to emergence	Resistance index		Days to emergence	Resistance index			
		Inoculated	Not inoculated		Inoculated	Not inoculated		Inoculated	Not inoculated	Inoculated	Not inoculated
Illinois No. 1:											
Madison, Wis	6	56.0	99.2	6	36.4	93.2	11	46.3	97.1	46.2	96.5
Indian Head, Saskatchewan	5	42.3	97.3	6	37.5	100.0	11	38.8	98.7	39.5	98.7
North Platte, Nebr	5	28.0	97.9	6	34.6	95.6	10	31.6	91.2	31.4	94.9
Felt, Idaho	6	20.0	94.1	6	15.9	89.8	10	22.3	96.0	19.4	93.3
Moccasin, Mont	5	19.2	91.5	6	17.0	89.4	10	15.8	89.9	17.3	90.3
Average	5	33.1	96.0	6	28.3	93.6	10	31.0	94.6	30.8	94.7
Marquis:											
Madison, Wis	9	30.0	96.0	9	25.6	95.5	12	39.0	98.7	31.5	96.7
North Platte, Nebr	5	32.5	91.9	6	24.9	96.3	11	28.8	94.2	28.7	94.1
Indian Head, Saskatchewan	6	18.7	98.3	6	15.7	97.5	12	18.8	100.0	17.7	98.6
Felt, Idaho	5	16.2	95.2	6	17.0	94.4	11	12.3	95.7	15.2	95.1
Moccasin, Mont	6	10.6	84.0	6	8.2	83.1	11	8.1	93.3	9.0	86.8
Moccasin, Mont.[1]	7	20.0	97.1	7	8.6	100.0	12	25.4	98.7	18.0	98.6
Average	6	21.3	93.8	7	16.7	94.5	12	22.1	96.8	20.0	95.0
Hard Federation:											
Madison, Wis	6	14.5	95.5	6	15.3	97.4	12	5.9	96.0	11.9	96.3
North Platte, Nebr	5	10.2	93.1	6	7.2	92.3	11	8.5	82.9	8.6	89.4
Indian Head, Saskatchewan	5	4.7	98.3	7	3.3	96.4	12	2.2	98.7	3.4	97.8
Moccasin, Mont	6	3.3	74.7	6	1.2	66.2	12	3.3	80.6	2.6	73.8
Felt, Idaho	6	2.9	89.9	7	2.1	96.2	12	2.5	86.5	2.5	90.9
Average	6	7.1	90.3	6	5.8	89.7	12	4.5	88.9	5.8	89.6
Peliss selection 89:											
North Platte, Nebr	9	21.1	73.1	9	20.8	73.9	11	20.3	91.3	20.7	79.4
Indian Head, Saskatchewan	11	17.5	88.8	11	10.0	93.2	12	12.8	99.6	13.4	93.9
Felt, Idaho	11	6.1	65.8	12	2.9	58.9	11	4.2	80.5	4.4	68.4
Moccasin, Mont	12	3.4	72.0	11	4.5	72.9	10	4.7	80.7	4.2	75.2
Average	11	12.0	74.9	11	9.6	74.7	11	10.5	88.0	10.7	79.2

BARLEY VARIETIES

Variety and station where seed was produced	Days to emergence	Inoculated	Not inoculated	Days to emergence	Inoculated	Not inoculated	Days to emergence	Inoculated	Not inoculated	Inoculated	Not inoculated
Hannchen selection 1:											
Madison, Wis	6	47.4	90.7	5	56.4	94.8	8	63.0	94.4	55.6	93.3
Indian Head, Saskatchewan	7	39.7	70.0	5	49.5	69.1	8	51.3	98.0	46.8	79.0
North Platte, Nebr	5	35.4	72.3	5	46.2	92.5	8	51.8	86.0	44.5	83.6
Felt, Idaho	5	39.3	67.2	5	36.7	88.8	8	46.4	91.5	40.8	82.5
Moccasin, Mont	8	23.6	80.3	5	39.2	80.5	8	42.0	84.7	34.9	81.8
Average	6	37.1	76.1	5	45.6	85.1	8	50.9	90.9	44.5	84.0
Trebi:											
Madison, Wis	11	73.4	93.5	6	66.3	88.3	9	77.7	95.9	72.5	92.6
North Platte, Nebr	7	42.1	95.5	6	43.6	97.5	9	49.6	84.3	45.1	92.4
Indian Head, Saskatchewan	8	35.9	97.1	6	43.1	96.3	9	51.2	98.8	43.4	97.4
Felt, Idaho	7	38.5	92.9	6	41.7	91.2	11	49.1	95.2	43.1	93.1
Moccasin, Mont	9	30.1	82.7	6	34.8	89.9	9	47.0	88.5	37.3	87.0
Average	8	44.0	92.3	6	45.9	92.6	9	54.9	92.5	48.3	92.5
Oderbrucker:											
Madison, Wis	5	66.0	95.2	5	71.7	95.6	9	78.3	97.3	72.0	96.0
North Platte, Nebr	5	50.3	94.1	5	46.3	97.3	9	58.3	95.5	51.6	95.6
Indian Head, Saskatchewan	5	48.9	94.9	5	45.6	97.1	9	47.9	98.7	47.5	96.9
Felt, Idaho	5	33.2	91.6	5	40.6	85.5	9	59.8	92.4	44.5	89.8
Moccasin, Mont	5	35.5	84.0	5	36.3	90.7	9	48.7	90.8	40.2	88.5
Average	5	46.8	92.0	5	48.1	93.2	9	58.6	94.9	51.2	93.4

[1] Harvested July 27 when grain was in early milk stage.
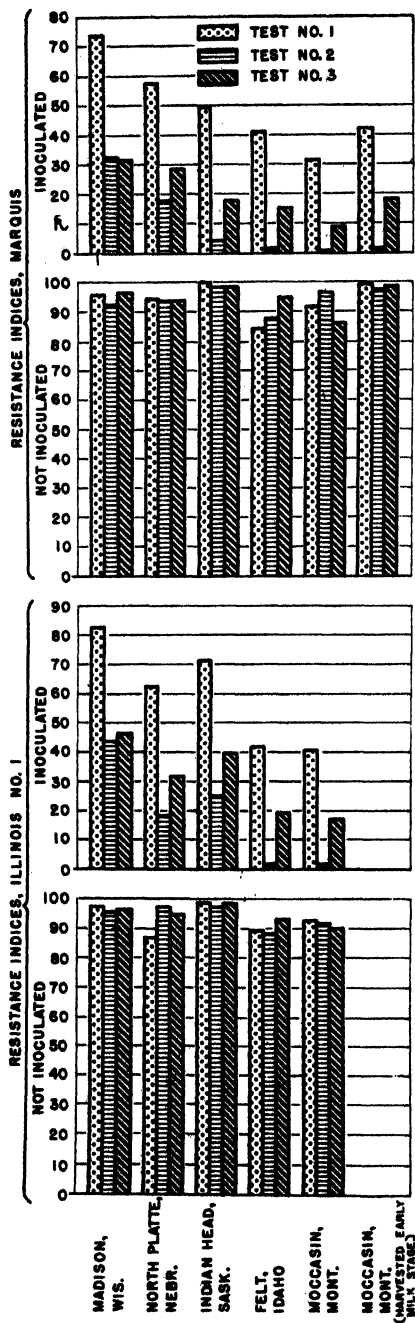
FIGURE 3.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of the wheat varieties Marquis and Illinois No. 1 grown in three tests in the greenhouse at Madison, Wis., from seed produced at each of five experiment stations in 1929.

The average resistance index for all the seed lots for the three temperatures was 30.8 for Illinois No. 1, 20.0 for Marquis, 10.7 for Peliss selection 89, and 5.8 for Hard Federation. The relative reaction of the varieties to seedling blight is the same as in tests 1 and 2 (table 3), except that Peliss selection 89 and Hard Federation are reversed. There was very little difference among the three barley varieties, although Hannchen selection 1 was slightly more susceptible in each test.

An interesting response of varieties to temperature during the period of germination is clearly shown by the results in table 5. When germinated at 12° C., Peliss selection 89 emerged in 11 days and Marquis in 12 days; but when germinated at 26°, Marquis emerged in 6 days, while Peliss selection 89 did not emerge until the eleventh day. Among the three barley varieties, high temperature had a similar retarding effect on Trebi. Apparently this response had no relation to seedling infection in these studies.

RESULTS WITH SEED PRODUCED IN 1930

The resistance indices for the seed lots of the 4 varieties of wheat and 3 varieties of barley produced at 12 experiment stations in 1930 are shown in table 6. Data for the four wheat varieties are shown in figure 4 and for the three barley varieties in figure 5. Four seedings, or a total of 500 kernels, of each seed lot of wheat were grown in inoculated soil; and 2 seedings, one of 75 and one of 100 kernels, were grown without inoculation. Two seedings, or a total of 225 kernels, of each seed lot of barley,

were grown in inoculated soil; while 2 seedings, or a total of 175 kernels, were grown without inoculation.

TABLE 6.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 4 varieties of wheat and 3 varieties of barley from seed produced at 12 experiment stations in 1930, when grown in the greenhouse at Madison, Wis.*

WHEAT VARIETIES

Station where seed was produced	Resistance index									
	Control (not inoculated)					Inoculated with *Gibberella saubinetii*				
	Illinois No. 1A	Marquis	Hard Federation	Peliss selection 89	Average	Illinois No. 1A	Marquis	Hard Federation	Peliss selection 89	Average
Indian Head, Saskatchewan	99.8	97.2	94.2	95.5	96.7	83.2	74.0	79.5	69.3	76.5
Arlington, Va	96.9	94.4	93.4	85.4	92.5	82.6	74.9	74.0	48.9	70.1
Bozeman, Mont	93.0	97.6	95.7	83.3	92.4	76.8	65.6	60.2	62.8	66.4
Madison, Wis	96.0	91.0	81.7	77.4	86.5	83.9	66.1	56.7	44.8	62.9
North Platte, Nebr	90.6	97.4	96.1	92.8	94.2	65.2	68.2	60.9	55.8	62.5
Felt, Idaho	88.2	79.3	71.4	79.6	79.6	74.1	63.7	55.0	55.3	62.0
Mandan, N. Dak	93.4	93.8	88.4	84.5	90.0	75.4	61.0	54.2	42.1	58.2
Aberdeen, Idaho	84.7	93.4	77.1	82.6	84.5	74.2	69.7	37.1	51.3	58.1
Moro, Oreg	83.7	85.2	93.3	91.8	88.5	63.0	57.6	48.6	50.6	55.0
Moccasin, Mont	90.7	83.8	85.4	91.1	87.8	60.9	58.7	38.5	51.2	52.3
Hays, Kans	87.7	90.4	90.8	52.3	80.3	46.9	64.0	59.2	21.0	47.8
Pendleton, Oreg	80.2	85.5	84.0	66.9	79.2	50.4	56.1	33.5	28.9	42.2
Average	90.4	90.8	87.6	81.9	87.7	69.7	65.0	54.8	48.5	59.5

BARLEY VARIETIES

Station where seed was produced	Resistance index							
	Control (not inoculated)				Inoculated with *Gibberella saubinetii*			
	Oderbrucker	Hannchen selection 1	Trebi	Average	Oderbrucker	Hannchen selection 1	Trebi	Average
Indian Head, Saskatchewan	94.3	98.7	96.7	96.6	89.3	80.7	92.7	87.6
Madison, Wis	97.2	88.8	93.4	93.1	93.0	80.7	88.8	87.5
Bozeman, Mont	97.3	94.4	95.0	95.6	86.9	89.2	79.0	85.0
Arlington, Va	97.4	91.0	95.6	94.7	84.0	91.1	78.1	84.4
Felt, Idaho	92.8	95.5	93.1	93.8	83.7	84.0	71.5	79.7
Aberdeen, Idaho	95.2	93.9	93.1	94.1	72.8	77.3	63.0	71.0
Hays, Kans	87.0	90.6	91.9	89.8	67.1	64.3	65.6	65.7
North Platte, Nebr	97.9	92.1	95.4	95.1	71.3	68.3	55.4	65.0
Mandan, N. Dak	99.0	99.1	93.5	97.2	59.2	70.6	64.7	64.8
Moro, Oreg	94.8	94.8	94.1	94.6	66.2	65.5	59.5	63.7
Moccasin, Mont	94.3	92.8	92.8	93.3	57.4	64.2	54.7	58.8
Pendleton, Oreg	94.0	91.3	94.9	93.4	51.4	61.6	52.0	55.0
Average	95.1	93.6	94.1	94.3	73.5	74.8	68.8	72.4

The viability and natural infection of the wheat that was not inoculated were more variable than in tests on the seed produced in 1929; yet after making allowance for this, marked differences in disease reaction of the same variety from seed produced at the different stations are apparent. Seed of most of the varieties of both wheat and barley from Indian Head, Madison, Arlington, and Bozeman showed higher resistance indices than that from Pendleton, Hays, Moccasin, and Moro. The similarity in the response for different
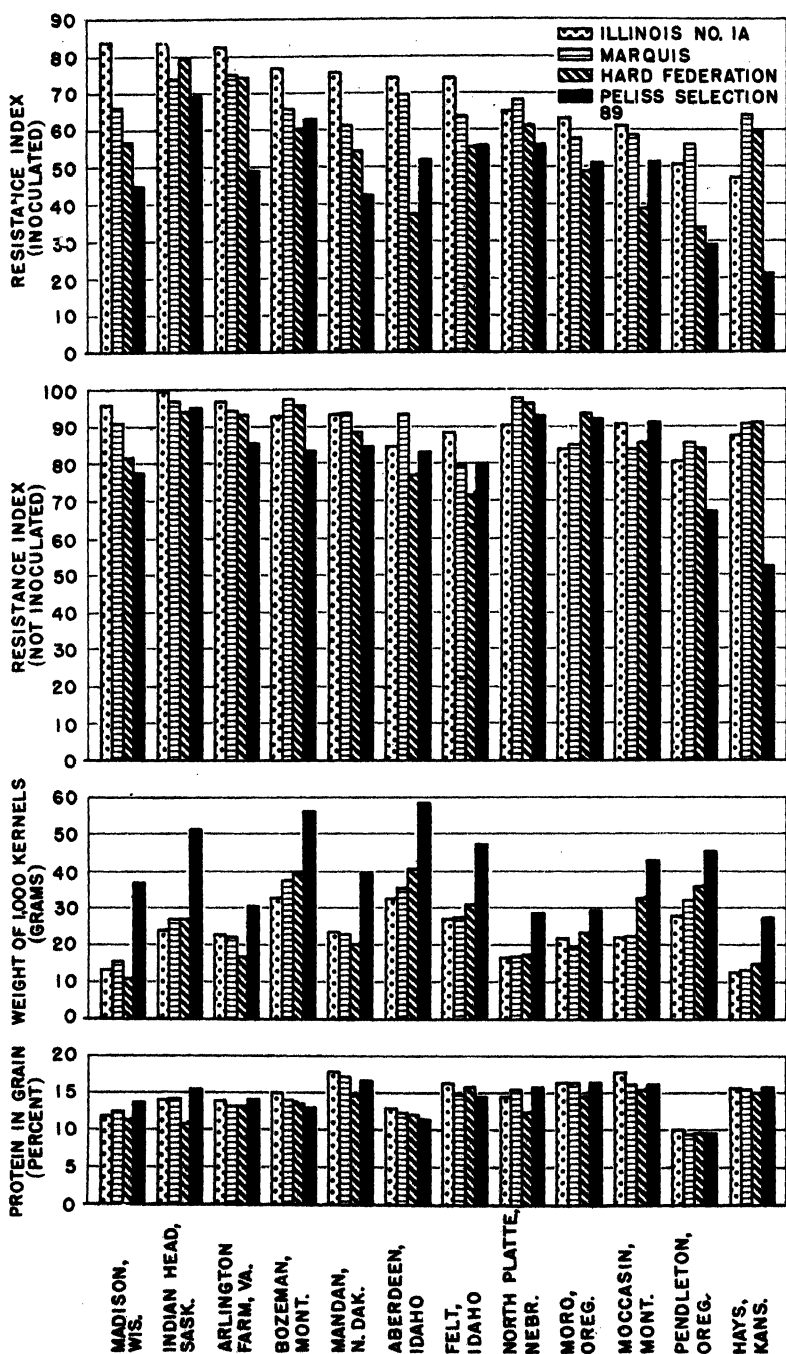
FIGURE 4.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of four varieties of wheat grown in the greenhouse at Madison, Wis., from seed produced at each of 12 experiment stations in 1930, and weight of 1,000 kernels and protein content of seed sown.
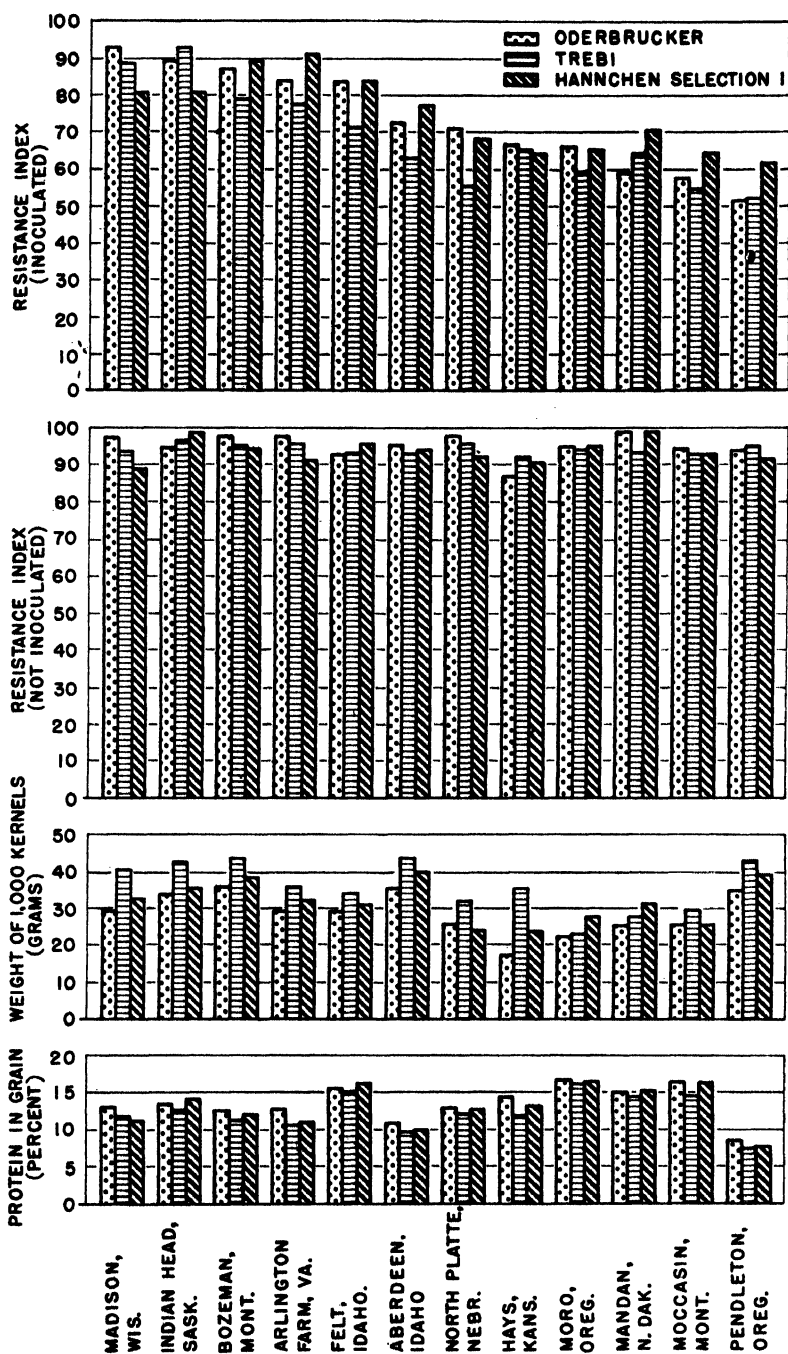
FIGURE 5.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of three varieties of barley grown in the greenhouse at Madison, Wis., from seed produced at each of 12 experiment stations in 1930, and weight of 1,000 kernels and protein content of seed sown.

varieties and the magnitude of the differences are such as to permit little opportunity for explaining the differences on the basis of random errors. For example, the uninoculated rows of Illinois No. 1A had resistance indices of 87.7 and 99.8 from seed produced at Hays and Indian Head, respectively, while in the inoculated series there was a spread from 46.9 to 83.2, or three times that in the control. Trebi produced at Indian Head and Pendleton had resistance indices of 96.7 and 94.9, respectively, when not inoculated; but for the same variety, when inoculated, the resistance indices were 92.7 and 52.0, respectively.

As an average for all stations, the resistance index of inoculated Illinois No. 1A was 69.7; Marquis, 65.0; Hard Federation, 54.8; and Peliss selection 89, 48.5. These resistance indices are relatively the same as for the seed produced in 1929. The differences between resistance indices for the three barley varieties were small, as were those for the 1929 seed.

Differences in the response of the varieties of wheat to environmental conditions are again indicated. For example, seed of Illinois No. 1A (inoculated) produced at Hays and Pendleton had resistance indices of 46.9 and 50.4, as compared with 64.0 and 56.1 for Marquis seed produced at these same stations; whereas, for seed produced at Madison the resistance index of Illinois No. 1 was 83.9 and that of Marquis was 66.1 (table 6 and fig. 4). Hard Federation seed produced at Hays shows a higher resistance index as compared with the other varieties than seed produced at the other stations, as was the case in 1929. Also a relatively lower resistance index was recorded for Hard Federation seed from Aberdeen than from other sources.

Seed lots of the three barley varieties from the several stations rank in about the same order for reaction to seedling blight (fig. 5), except that seed of Hannchen selection 1 from Indian Head and Madison was relatively more susceptible than the other two varieties. Seed of the varieties of wheat and barley produced at the several stations also ranked in about the same order at each station for weight of 1,000 kernels and protein content, although there are some cases in which the varieties show a differential response to environment. A notable example of this is the relatively large kernel weight of Trebi produced at Hays.

COMPARISON OF SEED PRODUCED IN 1929 AND 1930

Since it has been shown that varieties in some cases differ markedly in response to environment at the different stations and that averages of the varieties tend to obscure these differences, it seems best in comparing the seedling blight reaction of seed produced at the several stations in 1929 and 1930 to compare the varieties separately rather than to compare averages. Also, as mentioned earlier, the results from test no. 1 show the true range in disease reaction to inoculation more accurately than those from test no. 2, in the case of the wheat varieties produced in 1929; therefore only data from test no. 1 were used in this comparison. The average data from the two tests of barley were used.

The resistance indices of the seed of Illinois No. 1 and 1A wheat and Trebi barley produced at the several experiment stations in 1929

and 1930 are shown in figure 6. For the wheat the highest resistance index each year was that of seed produced at Madison and the lowest was that of seed produced at Hays; but resistance indices for seed from some stations, notably that from Bozeman, Aberdeen, Felt, and Mandan were relatively higher in 1930 than in 1929. The seed of Trebi barley from Indian Head and from Bozeman had a relatively higher resistance index in 1930 than in 1929, while that from North Platte had a relatively lower index. Similar results were obtained with the other varieties. For each of the varieties the protein content and especially the weight of 1,000 kernels produced at the several stations were similar for the 2 years. Apparently neither of these factors will account for the relative differences in reaction to seedling blight between the samples from the several stations for 2 years.

Although samples were obtained for only 2 years, it would seem that seed from some locations may be on the average more resistant to seedling blight over a period of years than seed from other locations but that in any one year the relative disease reaction may be reversed.

EFFECT OF PREMATURE HARVEST

The number of days from flowering, percentage of protein in the seed, weight of 1,000 kernels, and indices of resistance to seedling blight for the seed lots of Marquis wheat harvested at several stages of maturity at Moccasin in 1929 and 1930, are presented in table 7. The resistance indices for seed produced in 1929 are from duplicate inoculated rows and from one uninoculated row in each of two seedings in the greenhouse at Madison in January and February 1930. One hundred kernels were sown in each row. For the seed produced in 1930 there were 4 seedings, or a total of 500 kernels, of each lot grown in inoculated soil; and 2 seedings, one of 75 and one of 100 kernels, grown without inoculation. Tests on 1930 seed were conducted in the greenhouse at Madison, in January and February 1931.

TABLE 7.—*Weight of 1,000 kernels, percentage of protein, and indices of resistance to seedling blight of Marquis wheat harvested at several stages of maturity at Moccasin, Mont., in 1929 and 1930*

Date harvested [1]	Period from flowering to harvest	Protein	Weight of 1,000 kernels	Resistance index after indicated treatment of seed		Date harvested [1]	Period from flowering to harvest	Protein	Weight of 1,000 kernels	Resistance index after indicated treatment of seed	
				Not inoculated	Inoculated					Not inoculated	Inoculated
1929	*Days*	*Per-cent*	*Grams*			*1930*	*Days*	*Per-cent*	*Grams*		
July 27	11	15.2	9.0	98.6	21.5	July 15	7	------	5.5	94.4	67.7
Aug. 3	18	17.3	15.0	99.0	24.5	July 16	8	------	6.8	96.6	80.0
Aug. 9	24	17.9	16.3	92.1	22.5	July 17	9	------	8.0	97.3	81.8
Aug. 14	29	18.4	16.8	96.2	20.7	July 18	10	------	9.1	98.6	85.1
1930						July 19	11	------	10.6	98.6	80.1
						July 23	15	------	15.9	97.6	84.9
July 12	4	------	2.4	68.8	23.0	July 28	20	------	20.4	99.3	90.1
July 13	5	------	3.1	90.8	49.2	Aug. 1	24	------	21.5	97.6	90.3
July 14	6	------	4.3	90.4	64.9	Aug. 10	33	------	22.1	99.4	83.0

[1] Heads were tagged on July 16 and 17, 1929, and July 8, 1930, when anthers protruded from florets near the middle of the heads, and were harvested on the dates indicated.
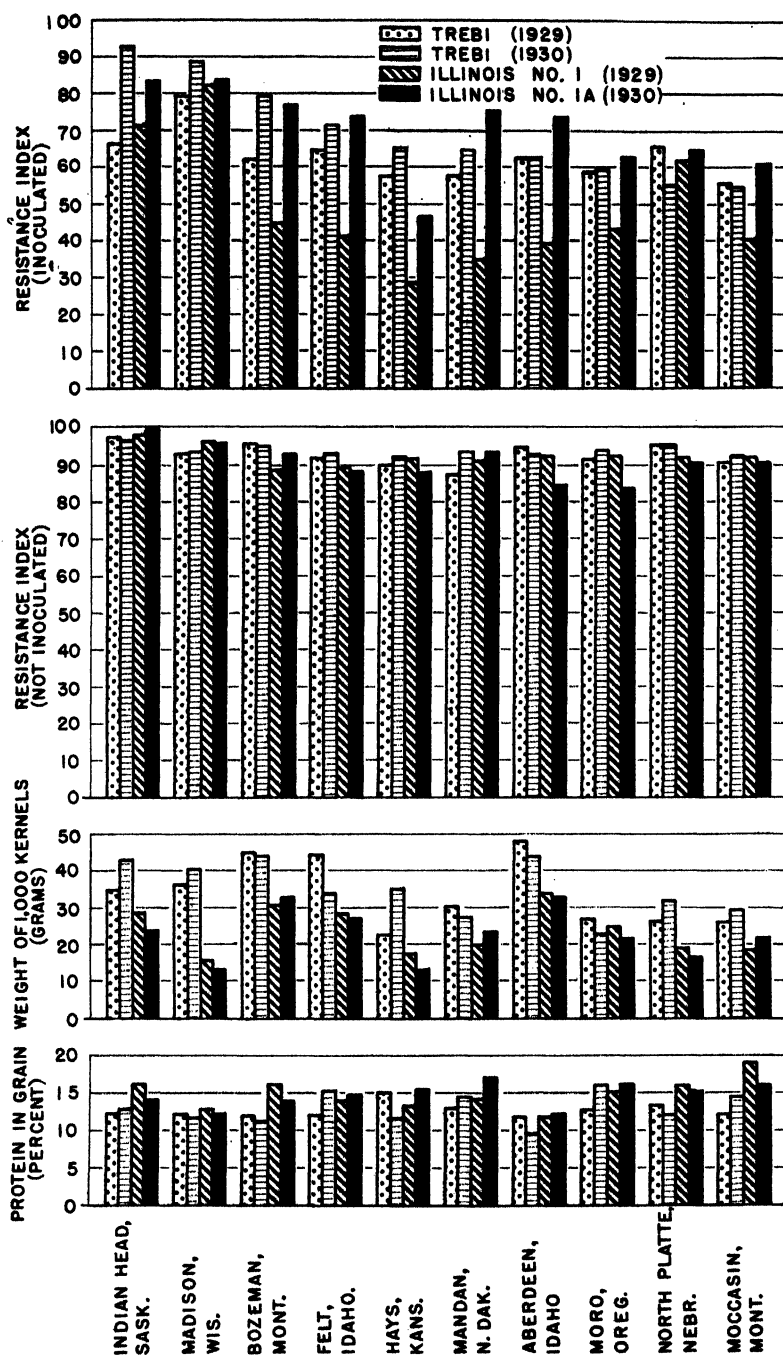
FIGURE 6.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of Trebi barley and Illinois No. 1 or Illinois No. 1A wheat, grown in the greenhouse at Madison, Wis., from seed produced at each of 10 experiment stations in 1929 and 1930, and weight of 1,000 kernels and protein content of seed sown.

Although the weight of 1,000 kernels of the 1929 crop ranged from 9.0 to 16.8 g, depending on the date of harvest, there were no significant differences in disease reaction of inoculated seed. Likewise, seed harvested on July 18, 1930, 10 days after flowering, which weighed only 9.1 g per 1,000 kernels, had a resistance index, when inoculated, equal to that of mature seed, which weighed 22.1 g per 1,000 kernels. On the other hand, seed harvested from the fourth to the seventh day after flowering was much more susceptible. This seed ranged in weight from 2.4 to 5.5 g per 1,000 kernels, and for this group the resistance index increased with the weight of the seed. The fact that kernels harvested only 11 days after blooming in 1929 and 10 days after blooming in 1930 had resistance indices equal to those of mature seed suggests that the differences in disease reaction noted for seed produced at different stations were not due to degree of maturation of the kernels. This also is indicated by the fact that seed badly shriveled by premature ripening at some stations (Madison and North Platte) was more resistant than plump, well-developed seed of the same variety from other stations (Aberdeen and Pendleton).

EFFECT OF SEED SIZE AND PROTEIN CONTENT

There is some suggestion in the data presented (figs. 1, 2, 4, 5) that reaction to seedling blight might be dependent upon or related to the protein content and size of the seed sown. In order to study this relation more critically, the correlation coefficients for indices of resistance and these two characters were calculated. The correlation coefficients for protein content and size of seed were also calculated in order to determine the partial correlation coefficients. It was observed, as may be noted in figures 4 and 5, that the seed lots from Pendleton were the lowest in protein content and among the highest in weight of 1,000 kernels; yet they showed the lowest resistance indices for each of the barley varieties and for 3 of the 4 wheat varieties. Since this relation was different, it appeared that the seed differed from that from other stations in some important but unknown respect, and correlation coefficients for 1930 were also calculated with the omission of Pendleton results. Correlation coefficients were first determined for each variety separately. Since they did not differ significantly for the barley varieties or for the wheat varieties except Hard Federation, a single correlation coefficient was calculated for the wheat varieties Marquis, Illinois No. 1, and Peliss selection 89, and for the barley varieties Trebi, Hannchen selection 1, and Oderbrucker. The correlation for the combined varieties was calculated from the z values of the correlations for the single varieties in order to correct for the means, which differed for the varieties. The correlation coefficients for the three varieties combined and those for each variety individually, as well as the means for the characters correlated, are given in table 8 for the seed lots produced in 1929 and in 1930. Partial correlation coefficients are given in table 9.

TABLE 8.—*Coefficients of correlation between indices of resistance to seedling blight, percentage of protein, and weight of 1,000 kernels seeded, and means for the 3 characters for varieties of wheat and barley from seed produced at several experiment stations in 1929 and 1930*

Year seed was produced and variety	Seed lots	Means			Resistance with protein		Resistance with kernel weight		Protein with kernel weight	
		Resist-ance in-dices	Pro-tein in grain	Weight of 1,000 kernels	Corre-lation coeffi-cient	P value	Corre-lation coeffi-cient	P value	Corre-lation coeffi-cient	P value
1929	*Num-ber*		*Per-cent*	*Grams*						
Hard Federation	10	33.6	14.5	27.4	+0.0486	0.89	−0.7222	0.02	−0.3018	0.40
Marquis	14	43.8	16.0	21.2	−.4992	.07	−.0148	.96	−.3873	.18
Illinois No. 1	10	48.9	15.0	23.7	+.0824	.82	−.1661	.65	−.1273	.73
Peliss selection 89	9	30.2	14.9	44.3	−.0100	.98	+.2331	.55	−.8174	<.01
3 wheat varieties	33				−.2257	.26	+.0037	.99	−.4713	<.01
Hannchen selection 1	10	60.1	13.7	29.0	−.6674	.04	+.1652	.65	−.4684	.18
Oderbrucker	10	62.4	14.0	26.2	−.7323	.02	+.3175	.37	−.4378	.21
Trebi	10	63.3	12.7	34.1	−.2571	.47	+.3242	.36	−.7171	.02
3 barley varieties	30				−.5834	<.01	+.2704	.20	−.5555	<.01
1930 [1]										
Hard Federation	12	54.8	13.1	25.7	−.0339	.92	−.4930	.10	−.0659	.84
Marquis	12	65.0	14.2	24.3	−.1089	.74	+.0386	.91	−.4217	.18
Illinois No. 1A	12	69.7	14.6	23.1	−.0113	.93	+.2108	.51	−.0751	.82
Peliss selection 89	12	48.5	14.3	41.0	+.1896	.56	+.4547	.14	−.5057	.09
3 wheat varieties	36				+.0238	.90	+.2427	.20	−.3458	.07
Hannchen selection 1	12	74.8	13.0	31.7	−.1130	.73	+.4416	.16	−.6288	.03
Oderbrucker	12	73.6	13.5	28.5	+.0158	.96	+.3448	.28	−.6450	.03
Trebi	12	68.8	12.3	35.8	+.0008	.99	+.4522	.15	−.7852	<.01
3 barley varieties	36				−.0323	.87	+.4139	.02	−.6937	<.01
1930 [2]										
Hard Federation	11	56.7	13.4	24.8	−.4241	.20	−.4081	.22	+.1584	.64
Marquis	11	65.8	14.6	23.6	−.6425	.04	+.2165	.52	−.2983	.37
Illinois No. 1A	11	71.5	15.0	22.7	−.4641	.16	+.3806	.25	+.0880	.08
Peliss selection 89	11	50.3	14.7	40.6	−.2321	.49	+.5770	.07	−.6370	.04
3 wheat varieties	33				−.4625	.02	+.4021	.04	−.3133	.12
Hannchen selection 1	11	76.0	13.5	31.0	−.4499	.17	+.7372	<.01	−.4722	.15
Oderbrucker	11	75.5	14.0	28.0	−.4855	.14	+.6203	.04	−.6017	.05
Trebi	11	70.3	12.7	35.2	−.3269	.33	+.6533	.03	−.7859	<.01
3 barley varieties	33				−.4230	.03	+.6735	<.01	−.6389	<.01

[1] Data from all stations included.
[2] Data from Pendleton, Oreg., omitted.

TABLE 9.—*Partial correlation coefficients between indices of resistance to seedling blight and protein content and weight of 1,000 kernels seeded, for seed lots of wheat and barley produced at several experiment stations in 1929 and 1930*

Year seed was produced and variety	Resistance with pro-tein (kernel weight held constant)		Resistance with ker-nel weight (protein content held con-stant)	
	Partial corre-lation coeffi-cient	P	Partial corre-lation coeffi-cient	P
1929				
3 wheat varieties	−0.2540	0.21	−0.1195	0.56
Hard Federation wheat	−.2567	.51	−.7430	.02
3 barley varieties	−.5411	<.01	−.0795	.72
1930 [1]				
3 wheat varieties	−.3870	.05	+.3055	.14
Hard Federation wheat	−.3988	.26	−.3813	.28
3 barley varieties	+.0128	.95	+.5784	<.01
1930 [2]				
3 wheat varieties	+.1183	.54	+.2675	.17
Hard Federation wheat	−.0765	.82	−.4965	.13
3 barley varieties	+.3886	.04	+.4839	.01

[1] Data from Pendleton, Oreg., omitted.   [2] Data from all stations included.

The correlation coefficients for resistance indices and protein content for wheat produced in 1929 are not significant for any variety taken separately nor for the three varieties considered together. The negative correlation (-0.4992, $P=0.07$) for Marquis approaches significance. Substantially the same is true for seed produced in 1930 when all stations are included. However, when Pendleton is omitted the correlation coefficients for all the varieties individually are negative and that for Marquis (-0.6425, $P=0.04$) and for the three wheat varieties combined (-0.4625, $P=0.02$) are significant.

Further light on the relationship between reaction to seedling blight and protein content is shown by the partial correlation coefficients given in table 9. When size of seed is held constant, Hard Federation shows a negative, though not statistically significant, partial correlation coefficient for protein content and resistance indices for seed produced in 1929 (-0.2567, $P=0.51$) and in 1930 (-0.3988, $P=0.26$), when Pendleton is omitted. The other three wheat varieties also show a small negative partial correlation coefficient (-0.2540, $P=0.21$) for seed lots produced in 1929 and a statistically significant negative partial correlation coefficient (-0.3870, $P=0.05$) for seed lots produced in 1930, when Pendleton is omitted.

The correlation coefficient for resistance indices and protein content for two of the three barley varieties for seed produced in 1929 is negative and significant (table 8). The coefficient for the third variety is also negative though not significant. For all three varieties taken together the coefficient is -0.5834, $P<0.01$. For the 1930 seed, when results from Pendleton are omitted all of the correlation coefficients for the varieties taken individually are negative, although none are significant. For all three varieties taken together the coefficient is -0.4230, $P=0.03$. Holding size of seed constant (table 9), a significant negative partial correlation coefficient (-0.5411, $P<0.01$) is obtained for the three barley varieties produced in 1929, while for the 1930 seed, with Pendleton omitted, the partial correlation coefficient is very small and not significant. It would appear from these data that a negative relation exists between protein content of the seed sown and resistance to seedling blight caused by *Gibberella saubinetii*, but that its influence may be overbalanced by other factors.

Since, as will be shown later, a high correlation ($+0.9312$, $P<0.01$ for wheat, and $+0.8213$, $P<0.01$ for barley) exists between size of seed and dry weight of seedlings, a high correlation might be expected between resistance to seedling blight and size of seed. In general, the data support this assumption but they are not entirely consistent. Thus the correlation coefficients for individual varieties are positive for 10 of the 14 variety years but are statistically significant only for barley varieties produced in 1930. No statistically significant correlations were found for the wheat varieties Marquis, Illinois No. 1, and Peliss selection 89 for seed lots produced in 1929. The correlation coefficient for seed of these varieties produced in 1930 is $+0.2427$, $P=0.20$, for all seed lots and $+0.4021$, $P=0.04$, when those from Pendleton are omitted. Hard Federation, however, shows a significant negative correlation coefficient (-0.7222, $P=0.02$) for seed produced in 1929 and a negative, though not statistically significant, correlation coefficient (-0.4081, $P=0.22$) for 1930 seed when Pendleton is omitted. Holding protein content constant, the partial correlation coefficients for Marquis, Illinois No. 1A, and Peliss selection

89 show little or no relation between size of seed and resistance indices for seed produced in either year. A significant negative partial correlation coefficient (-0.7430, $P=0.02$) was found for Hard Federation seed produced in 1929 and a negative but not statistically significant partial correlation coefficient (-0.3813, $P=0.28$) for 1930 seed. It does not seem reasonable that small seed as such is correlated with resistance indices in the Hard Federation variety but rather that some other condition closely associated with size of seed is concerned. The nature of this condition is not apparent from the present studies.

The three barley varieties show a positive but not statistically significant correlation coefficient ($+0.2704$, $P=0.20$) between size of seed and resistance to seedling blight for seed lots produced in 1929, and a significant positive correlation coefficient ($+0.4139$, $P=0.02$) for all seed lots produced in 1930. When data on the seed lots from Pendleton are omitted the correlation coefficient becomes $+0.6735$, $P<0.01$. When protein content is held constant, partial correlation coefficients show that size of seed in barley was not correlated with resistance indices in 1929 seed lots, but was rather high for 1930 seed lots when all stations were included ($+0.4839$, $P=0.01$) and when Pendleton was omitted ($+0.5784$, $P<0.01$). These data indicate that size of seed as well as protein content may sometimes influence the reaction of wheat and barley to seedling blight.

REACTION TO BUNT

Seed of Marquis, Hard Federation, Illinois No. 1, and Illinois No. 1A, produced at each of the several experiment stations, was inoculated with bunt spores and grown in duplicate 8-foot rows at Moccasin in 1930 and 1931, and at Madison in 1931. Results from these tests are given in table 10. A generalized standard error was calculated for each variety tested at each station, and the standard error of a difference for the averages was computed from these.

TABLE 10.—*Percentage of bunt in three varieties of spring wheat grown at Moccasin, Mont., and Madison, Wis., from seed produced at several experiment stations in 1929 and 1930*

Variety, year, and station on which seed was produced	Percentage of bunt at--				
	Moccasin, Mont.		Madison, Wis., 1931	Average, 1931	Average of 3 station years
	1930	1931			
Hard Federation:					
1929:					
Indian Head, Saskatchewan	93	83	79	81.0	85.0
Hays, Kans.	86	84	81	82.5	83.7
North Platte, Nebr.	87	89	70	79.5	82.0
Mandan, N. Dak.	90	77	77	77.0	81.3
Moro, Oreg.	78	72	76	74.0	75.3
Moccasin, Mont.	76	91	55	73.0	74.0
Bozeman, Mont.	77	81	59	70.0	72.3
Felt, Idaho.	76	77	57	67.0	70.0
Aberdeen, Idaho	65	82	60	71.0	69.0
1930:					
North Platte, Nebr.		82	82	82.0	
Mandan, N. Dak.		85	78	81.5	
Arlington, Va.		81	79	80.0	
Bozeman, Mont.		87	67	77.0	
Hays, Kans.		84	67	75.5	
Felt, Idaho.		75	73	74.0	
Madison, Wis.		76	70	73.0	
Moccasin, Mont.		74	69	71.5	
Moro, Oreg.		81	62	71.5	
Indian Head, Saskatchewan.		80	61	70.5	
Aberdeen, Idaho		77	62	69.5	
Pendleton, Oreg.		79	58	68.5	
Standard error of a difference				10.2	9.6

TABLE 10.—*Percentage of bunt in three varieties of spring wheat grown at Moccasin, Mont., and Madison, Wis., from seed produced at several experiment stations in 1929 and 1930*—Continued

Variety, year, and station on which seed was produced	Percentage of bunt at—				
	Moccasin, Mont.		Madison, Wis., 1931	Average, 1931	Average of 3 station years
	1930	1931			
Illinois No. 1:					●
1929:					
Hays, Kans	67				
Madison, Wis	50	90	22	56.0	54.0
North Platte, Nebr	52	75	35	55.0	54.0
Moccasin, Mont	50	70	37	53.5	52.3
Bozeman, Mont	52	69	17	43.0	46.0
Indian Head, Saskatchewan	46	65	27	46.0	46.0
Mandan, N. Dak	49	66	21	43.5	45.3
Felt, Idaho	39	68	26	47.0	44.4
Aberdeen, Idaho	41	66	23	44.5	43.3
Moro, Oreg	39	54	24	39.0	39.0
Standard error of a difference				9.3	8.3
Illinois No. 1A:					
1930:					
North Platte, Nebr		81	75	78.0	
Indian Head, Saskatchewan		86	62	74.0	
Felt, Idaho		86	49	67.5	
Bozeman, Mont		82	49	65.5	
Hays, Kans		82	49	65.5	
Arlington, Va		74	52	63.0	
Mandan, N. Dak		84	40	62.0	
Aberdeen, Idaho		77	44	60.5	
Moro, Oreg		66	48	57.0	
Moccasin, Mont		77	33	55.0	
Pendleton, Oreg		60	44	52.0	
Madison, Wis		66	24	45.0	
Standard error of a difference				9.3	
Marquis:					
1929:					
Moccasin, Mont. (July 27) [1]	37	54	11	32.5	34.0
Mandan, N. Dak	24	54	17	35.5	31.7
Moccasin, Mont. (Aug. 14) [1]	27	48	15	31.5	30.0
Madison, Wis	24	52	13	32.5	29.7
North Platte, Nebr	31	49	7	28.0	29.0
Moro, Oreg	22	55	9	32.0	28.7
Moccasin, Mont. (Aug. 3) [1]	30	48	7	27.5	28.3
Moccasin, Mont. (Aug. 9) [1]	26	45	12	28.5	27.7
Hays, Kans	30	46	3	24.5	26.3
Bozeman, Mont	16	48	6	27.0	23.3
Aberdeen, Idaho	19	44	4	24.0	22.3
Felt, Idaho	14	41	5	23.0	20.0
Indian Head, Saskatchewan	13	36	8	22.0	19.0
1930:					
Moro, Oreg		52	13	32.5	
North Platte, Nebr		52	13	32.5	
Madison, Wis		58	4	31.0	
Mandan, N. Dak		47	10	28.5	
Indian Head, Saskatchewan		46	6	26.0	
Arlington, Va		42	8	25.0	
Felt, Idaho		43	3	23.0	
Hays, Kans		42	4	23.0	
Bozeman, Mont		38	4	21.0	
Aberdeen, Idaho		38	2	20.0	
Pendleton, Oreg		35	4	19.5	
Moccasin, Mont.:					
Aug. 10 [1]		36	4	20.0	
Aug. 1 [1]		38	5	21.5	
July 28 [1]		28			
July 23 [1]		29	4	16.5	
July 19 [1]		38	2	20.0	
July 18 [1]		52	2	27.0	
July 17 [1]		28	6	17.0	
July 16 [1]		45	2	23.5	
July 15 [1]		51	4	27.5	
July 14 [1]		45	3	24.0	
Standard error of a difference				5.9	6.1

[1] Heads were tagged at Moccasin, Mont., on July 16, 1929, and July 8, 1930, when anthers were protruded from florets near the center of the heads, and were harvested on the dates indicated.

No significant differences in bunt infection were observed for Illinois No. 1 produced at the several experiment stations in 1929 nor for Hard Federation produced in either year. A difference of 33.0 percent in infection was recorded for the seed lots of Illinois No. 1A produced at North Platte (78.0 percent), and at Madison (45.0 percent), in 1930. This difference (3.5 times its standard error) was the only one for this variety that was more than twice the standard error. As an average for the three tests, the Marquis wheat produced at Felt and at Indian Head showed less bunt infection than seed harvested July 27 at the early milk stage at Moccasin, the differences being 2.3 and 2.5 times the standard error respectively. Considering the data for the seed lots of Marquis produced at the several experiment stations, when grown in the tests at Moccasin and Madison in 1931, the greatest difference is between that produced at Mandan in 1929 and that produced at Pendleton in 1930. The average difference in infection is 16.0 percent, which is 2.7 times the standard error. There are no statistically significant differences between seed lots of Marquis harvested at several stages of maturity at Moccasin, although there is a tendency for the seed lots with smaller kernels to be more susceptible.

A check row of Marquis from seed produced at Aberdeen in 1930 was sown after each nine seed lots in the bunt tests conducted in 1931. The greatest difference in bunt infection between any two checks was 12, which is 2.6 times the standard error. Considering the number of comparisons and the variability in the checks, it seems doubtful if any of the differences are really significant, except possibly the difference between the two seed lots mentioned of Illinois No. 1A.

A further test was made by calculating correlation coefficients for weight of 1,000 kernels sown and the average percentage of bunt in the tests at Moccasin and Madison in 1931. The correlation coefficients were -0.5358, $P=0.01$, for 21 lots of Hard Federation; -0.1861, $P=0.42$, for 21 lots of Illinois No. 1 and No. 1A combined; and -0.2143, $P=0.23$, for 33 lots of Marquis. When the coefficients for the three varieties were combined, a small but statistically significant coefficient (-0.3036, $P=0.01$) was obtained. These coefficients would seem to indicate that size of seed sown does affect the reaction of a given variety to bunt. That it is not the only factor nor a very important one is evidenced by the small and in most cases nonsignificant correlation coefficient and by the fact that seed of Illinois No. 1A produced at Madison had the lowest bunt infection of any seed lot of this variety and yet had the smallest kernels.

VIGOR OF SEEDLINGS AND YIELD OF GRAIN

Yield tests were conducted in 1930 and 1931 at Moccasin, where each seed lot was grown in four nursery plots; in 1930 and 1931 at Madison; and in 1931 at Aberdeen. At Madison and Aberdeen each seed lot was grown in three nursery plots. Approximately 400 kernels were sown in each 18-foot row, so that stands were about equal for each seed lot. Seeding was done at the normal seeding time at each station. At each station 16 feet from the center row of each three-row plot was harvested for yield. The dry weight of 100 seedlings at about the five-leaf stage from each seed lot was also determined from healthy plants in the uninoculated rows grown with the seedling

blight tests in the greenhouse at Madison. The weight of 1,000 kernels of the seed sown, the dry weight of the seedlings, and the yield in 1930 of the seed lots produced in 1929 are given in table 11. In addition to the above-mentioned tests, seed of three varieties of wheat and one of barley produced at each of four stations in 1929 was again tested for yield in 1931 at Madison, Moccasin, and Aberdeen, together with seed of the same varieties produced at the same stations in 1930. The results are presented in table 12.

TABLE 11.—*Yields at Madison, Wis., and Moccasin, Mont., in 1930, of the seed lots of 4 varieties of wheat and 3 of barley, produced at 10 experiment stations in 1929, weight of 1,000 kernels of the seed sown, and dry weight of 100 seedlings at fifth-leaf stage in the greenhouse at Madison, Wis.*

WHEAT VARIETIES

Variety and station where seed was produced	Weight of 1,000 kernels sown	Dry weight of 100 seedlings	Yield of grain per 16-foot row		
			Moccasin, Mont.	Madison, Wis.	Average
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Marquis:					
Moccasin, Mont.[1][2]	18.0	2.78	139	179	159
Do.[1][3]	15.0	2.37	162	156	159
Felt, Idaho	32.8	4.06	121	196	159
Indian Head, Saskatchewan	29.0	3.92	136	178	157
Mandan, N. Dak	19.2	2.72	138	172	155
Aberdeen, Idaho	35.1	4.10	129	179	154
Moccasin, Mont.[1][4]	16.3	2.65	163	138	151
Bozeman, Mont	34.8	4.39	129	168	149
Moro, Oreg	20.2	2.76	132	157	145
Hays, Kans	16.6	2.29	128	151	140
Moccasin, Mont.[1][5]	9.0	1.48	151	120	136
Madison, Wis	14.8	2.01	125	144	135
North Platte, Nebr	19.4	2.92	123	145	134
Illinois No. 1:					
Madison, Wis	15.8	2.30	147	161	154
Bozeman, Mont	30.5	4.47	156	142	149
Felt, Idaho	28.6	4.24	132	158	145
Aberdeen, Idaho	34.0	4.48	140	145	143
Hays, Kans	17.4	2.72	107	153	130
Indian Head, Saskatchewan	28.5	4.22	136	121	129
Moccasin, Mont	18.4	3.16	137	121	129
Mandan, N. Dak	20.0	3.02	137	105	121
North Platte, Nebr	19.0	3.22	126	109	118
Moro, Oreg	24.8	2.72	129	93	111
Peliss selection 89:					
Moccasin, Mont	29.7	3.17	176	309	243
Aberdeen, Idaho	61.1	4.55	180	269	225
Bozeman, Mont	60.6	4.85	169	264	217
Moro, Oreg	37.0	3.04	176	247	212
Felt, Idaho	55.2		162	253	208
Indian Head, Saskatchewan	52.4	4.38	179	225	202
North Platte, Nebr	39.1	3.63	171	214	193
Mandan, N. Dak	36.3	3.23	173	213	193
Hays, Kans	27.0	2.58	180	205	193
Hard Federation:					
Aberdeen, Idaho	39.8	3.21	147	104	126
Indian Head, Saskatchewan	34.8	3.59	151	100	126
Hays, Kans	18.2	2.88	142	103	123
Bozeman, Mont	35.6	3.72	137	99	118
Mandan, N. Dak	23.0	3.22	134	97	116
Felt, Idaho	35.6	3.81	127	96	112
Moro, Oreg	28.9	3.29	134	88	111
North Platte, Nebr	18.8	2.25	132	86	109
Moccasin, Mont	24.2	2.95	129	85	107
Madison, Wis	15.2	1.93	92	70	81
Standard error of a difference			19.3	28.2	24.5

[1] Heads were tagged on July 8, when anthers were protruded from florets near the center of the heads, and harvested at the indicated stage of development.
[2] Mature stage.
[3] Soft-dough stage.
[4] Hard-dough stage.
[5] Early milk stage.

TABLE 11.—*Yields at Madison, Wis., and Moccasin, Mont., in 1930, of the seed lots of 4 varieties of wheat and 3 of barley, produced at 10 experiment stations in 1929, weight of 1,000 kernels of the seed sown, and dry weight of 100 seedlings at fifth-leaf stage in the greenhouse at Madison, Wis.*—Continued

BARLEY VARIETIES

Variety and station where seed was produced	Weight of 1,000 kernels sown	Dry weight of 100 seedlings	Yield of grain per 16-foot row		
			Moccasin, Mont.	Madison, Wis.	Average
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Hannchen selection 1:					
Felt, Idaho	37.7	4.71	172	355	264
Hays, Kans	17.2	2.45	173	322	248
Indian Head, Saskatchewan	31.2	3.74	150	338	244
Moccasin, Mont	23.2	3.14	163	315	239
North Platte, Nebr	19.2	2.90	148	329	239
Madison, Wis	29.4	3.25	152	321	237
Bozeman, Mont	38.3	3.53	196	275	236
Mandan, N. Dak	26.3	2.78	185	284	235
Aberdeen, Idaho	42.8	3.93	170	297	234
Moro, Oreg	24.9	2.67	173	277	225
Oderbrucker:					
Bozeman, Mont	37.2	4.97	153	395	274
Aberdeen, Idaho	37.2	4.80	115	376	246
North Platte, Nebr	20.3	3.09	132	323	228
Hays, Kans	16.3	2.04	118	327	223
Indian Head, Saskatchewan	26.0	3.46	131	306	219
Moro, Oreg	20.2	3.31	109	301	205
Felt, Idaho	33.7	6.74	147	260	204
Mandan, N. Dak	25.7	3.09	122	269	196
Madison, Wis	26.1	3.84	110	271	191
Moccasin, Mont	19.5	3.33	105	265	185
Trebi:					
Aberdeen, Idaho	48.1	3.91	196	586	391
Madison, Wis	36.5	4.56	218	549	384
Indian Head, Saskatchewan	34.7	3.25	241	515	378
Bozeman, Mont	44.9	3.99	216	487	352
Hays, Kans	22.6	2.66	185	506	346
North Platte, Nebr	26.5	3.08	204	479	342
Mandan, N. Dak	30.6	3.34	201	465	333
Felt, Idaho	44.2	4.24	197	455	326
Moccasin, Mont	26.2	2.45	212	428	320
Moro, Oreg	27.1	2.78	166	447	307
Standard error of a difference			25.2	48.9	39.6

TABLE 12.—*Yields of seed lots of 1 variety of barley and 3 varieties of wheat, produced at 4 experiment stations in 1929 and 1930, when grown at Madison, Wis., Moccasin, Mont., and Aberdeen, Idaho, in 1930 and 1931, and weight of 1,000 kernels of seed sown*

Variety and station where seed was produced	Year seed was produced	Weight of 1,000 kernels sown	Yield of grain per 16-foot row						
			Moccasin, Mont., 1930	Madison, Wis., 1930	Moccasin, Mont., 1931	Madison, Wis., 1931	Aberdeen, Idaho, 1931	Average (3 stations 1931)	Average (5 station years)
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Trebi barley:									
Aberdeen, Idaho	1929	48.1	196	586	150	330	512	331	355
	1930	43.8			170	342	495	336	
Indian Head, Saskatchewan	1929	34.7	241	515	126	303	503	311	338
	1930	42.7			173	350	532	352	
North Platte, Nebr	1929	26.5	204	479	131	301	435	289	310
	1930	31.8			139	337	525	334	
Moccasin, Mont	1929	26.2	212	428	125	315	492	311	314
	1930	29.8			131	334	502	322	
Standard error of a difference			25.2	48.9	19.3	16.3	35	35.6	38.5

TABLE 12.—*Yields of seed lots of 1 variety of barley and 3 varieties of wheat, produced at 4 experiment stations in 1929 and 1930, when grown at Madison, Wis., Moccasin, Mont., and Aberdeen, Idaho, in 1930 and 1931, and weight of 1,000 kernels of seed sown*—Continued

Variety and station where seed was produced	Year seed was produced	Weight of 1,000 kernels sown	Yield of grain per 16-foot row						
			Moccasin, Mont., 1930	Madison, Wis., 1930	Moccasin, Mont., 1931	Madison, Wis., 1931	Aberdeen, Idaho, 1931	Average (3 stations 1931)	Average (5 station years)
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Hard Federation wheat:									
Indian Head, Saskatchewan	1929	34.8	151	100	99	100	420	206	174
	1930	27.1	....	....	79	101	388	189	....
Aberdeen, Idaho	1929	39.8	147	104	115	91	362	189	164
	1930	40.3	....	....	107	97	457	220	....
Moccasin, Mont	1929	24.2	129	85	85	72	377	178	150
	1930	32.4	....	....	100	116	443	220	....
North Platte, Nebr	1929	18.8	132	86	93	74	365	177	150
	1930	17.5	....	....	97	80	447	208	....
Peliss selection 89 wheat:									
Moccasin, Mont	1929	29.7	176	309	131	116	448	232	236
	1930	42.5	....	....	142	110	410	221	....
Indian Head, Saskatchewan	1929	52.4	179	225	119	135	440	231	220
	1930	51.2	....	....	128	116	462	235	....
Aberdeen, Idaho	1929	61.1	180	269	....	....	420	....	....
	1930	58.3	....	....	125	122	438	228	....
North Platte, Nebr	1929	39.1	171	214	103	156	400	220	209
	1930	28.5	....	....	103	118	435	219	....
Marquis wheat:									
Aberdeen, Idaho	1929	35.1	129	179	91	82	450	208	186
	1930	35.3	....	....	115	99	392	202	....
Indian Head, Saskatchewan	1929	29.0	136	178	96	87	388	190	177
	1930	27.1	....	....	91	96	352	180	....
North Platte, Nebr	1929	19.4	123	145	81	92	435	203	175
	1930	17.0	....	....	93	92	338	174	....
Moccasin, Mont.[1][2]	1929	18.0	139	179	75	84	373	177	170
Do.[1][3]	1929	16.3	163	138	103	94	373	190	174
Do.[1][4]	1929	15.0	162	156	89	96	400	195	181
Do.[1][5]	1929	9.0	151	120	83	81	400	188	167
Do.[1][6]	1930	22.3	....	....	83	85	333	167	....
Do.[1][7]	1930	21.0	....	....	97	....	363	....	....
Do.[1][8]	1930	9.2	....	....	84	74	320	159	....
Do.[1][9]	1930	5.5	....	....	84	80	258	141	....
Standard error of a difference	....	....	19.3	28.2	16.3	7.4	47.4	29.7	28.2

[1] Heads were tagged on July 16, 1929, and July 8, 1930, when anthers were protruded from florets near the middle of the heads, and harvested on the dates indicated.
[2] Mature stage.
[3] Hard-dough stage.
[4] Soft-dough stage.
[5] Early milk stage.
[6] Aug. 10.
[7] Seed sown at Moccasin and Aberdeen; harvested Aug. 1 and July 28, respectively.
[8] Seed sown at Moccasin, Madison, and Aberdeen; harvested July 19, 17, and 18, respectively.
[9] Seed sown at Moccasin, Madison, and Aberdeen; harvested July 16, 15, and 14, respectively.

Only 1 of the 57 seed lots of wheat and 2 of the 34 seed lots of barley gave yields that differed by more than twice the standard error from any other lot of the same variety. Seed of Peliss selection 89 produced at Moccasin in 1929 yielded more than seed from Hays, Mandan, and North Platte by as much as twice the standard error of the difference when only the average yields from the two tests in 1930 are considered. However, the seed from Moccasin and North Platte was included in the three tests in 1931, and the average yields reported in table 13 do not differ significantly. The yield of Marquis harvested on July 14, 15, and 16, 1930, when the weight of 1,000 kernels was only 5.5 g, was less than that of the highest yielding seed lot, which was produced at Aberdeen in 1929, the difference being only 2.3 times the standard error. Seed of Oderbrucker barley from Bozeman in 1929, yielded

more than seed produced at Moccasin by 2.2 times the standard error, and the difference between the yield of Trebi from Aberdeen and Moro in 1929, was 2.1 times the standard error. The seed of Oderbrucker from Bozeman and of Trebi from Aberdeen each weighed more per 1,000 kernels than other seed lots of the same variety. Differences in yield other than for the three seed lots mentioned are no greater than can be explained by random variation, and possibly these three should not be considered statistically significant inasmuch as the differences for the Marquis samples are based on yield tests at only three stations in 1931 and those for Trebi and Oderbrucker on yield tests at only two stations in 1930. It would be expected that in testing 91 seed lots some would be found which differ by more than twice the standard error owing to random variation alone.

Correlation coefficients were calculated for the weight of 1,000 kernels of the seed sown and the average grain yield, and for the weight of 1,000 kernels sown and dry weight of the seedlings at about the fifth-leaf stage. Correlation coefficients were first calculated for each variety, and since no significant differences were shown they were calculated for all varieties taken as a whole. The correlation coefficients are given in table 13.

TABLE 13.—*Coefficients of correlation for weight of 1,000 kernels sown and dry weight of 100 seedlings and yield of grain of wheat and barley varieties*

		Weight of 1,000 kernels sown correlated with—			
		Dry weight of 100 seedlings		Yield of grain	
Year and variety tested for yield	Seed lots	Correlation coefficient	P	Correlation coefficient	P
1930	*Number*				
Hard Federation	10	+0.8422	<0.01	+0.6082	0.07
Peliss selection 89	9	+.8801	<.01	+.1545	.69
Marquis	13	+.9797	<.01	+.4685	.11
Illinois No. 1	10	+.8986	<.01	+.2637	.46
4 varieties of wheat	42	+.9312	<.01	+.4026	.02
Hannchen selection 1	10	+.8201	<.01	+.1166	.75
Oderbrucker	10	+.8241	<.01	+.5521	.10
Trebi	10	+.8197	<.01	+.5004	.15
3 varieties of barley	30	+.8213	<.01	+.4049	.05
1931					
Trebi	8			+.7093	.05
Hard Federation	8			+.4498	.27
Peliss selection 89	7			+.4400	.33
Marquis	13			+.6535	.02
4 varieties	36			+.5978	<.01

The correlation coefficient for size of seed sown and grain yield was +0.4026, P=0.02 for wheat and +0.4049, P=0.05 for barley. Correlation coefficients for size of seed sown and yield were also calculated for the average yield at three stations in 1931 of one variety of barley and three varieties of wheat. Only seed lots from the stations which gave the maximum differences in yield in 1930 were included in these tests and, as would be expected, a higher correlation coefficient (+0.5978, P<0.01) was obtained.

The correlation coefficient for weight of seed and dry weight of seedlings for the four varieties of wheat was +0.9312, P<0.01, and for the three varieties of barley +0.8213, P<0.01. As would be

expected, the correlation is much greater between size of seed sown and dry weight of seedlings than between size of seed sown and grain yield. The effects of size of seed on vigor diminish as the plant approaches heading and maturity.

DISCUSSION

It has been shown that the environmental conditions under which seed of a variety of wheat or barley is produced may markedly affect the reaction of plants grown from it to seedling blight caused by *Gibberella saubinetii*. Seedling blight reaction was found to• be influenced somewhat by protein content and size of seed sown. The data indicate that size of seed may have a slight influence on reaction to bunt caused by *Tilletia levis*. The effect on grain yield (when about equal numbers of kernels were seeded per row) was not pronounced, although differences of more than twice the standard error were obtained and a correlation between size of seed sown and yield was shown.

Such differences are not due to changes in the hereditary factors of the plant (i. e., the genes) but are simply differences in the expression of these factors resulting from environment. The optimum for each environmental factor for plant growth, such as light or temperature, varies as the other factors change, and the result is a complex interaction of all the factors that govern the expression of plant characters. In addition to the interaction of all the environmental factors at any stage of development, the physiological balance in the plant carried over from the preceding growth phase or from the embryo may influence development. The effect of environment on the seed while it is attached to the mother plant may be expressed in the developing seedling as a result of variation in the nutrients from the endosperm or as a direct result of variations in the reserve food and physiologic balance in the embryo. Maturation of seed is relative insofar as the condition of the embryo and endosperm is concerned, and, in wheat grown in the field, may take place as soon as 3 weeks or as late as 9 weeks after fertilization. It is a dormant condition of the embryo brought about by desiccation and is not definite as to the stage of development of the embryo or endosperm. Deming and Robertson (*2*) have shown that in the seed of some varieties a rest period after harvest is necessary before growth can be resumed but that in other varieties growth can be resumed immediately after or even before translocation of materials from the mother plant to the seed has ceased. In the small grains, dormancy during the period between germination and maturity is usually not so complete as is the dormancy of the seed, but here again the difference is only relative, as winter wheat plants thoroughly hardened during periods of low temperature respire very slowly. On the other hand, the respiration of seeds may be very slow under conditions of extreme desiccation and low temperatures, or may be very rapid under conditions favorable for germination. It would seem, then, that the effect on the subsequent crop of the environment under which seed is produced is directly comparable to the effect of environment during one stage of plant growth on subsequent stages; and it is generally recognized that plants transplanted to a widely different environment will not respond in the same manner as plants grown continuously in the same environment.

Dickson (*4*) has shown that the *Gibberella* parasite enters the seedling plant through natural wounds, at root ruptures, by means of mechanical injuries, and by direct penetration of the cortical tissues, as well as through the endodermis into the central cylinder of roots. In resistant plants, a suberinlike substance is deposited in the walls of cells around these ruptured tissues and the outer cortical cells, which retards or prevents penetration of the fungus. Infection takes place in wheat and barley seedlings prior to or very soon after emergence. It might be expected that a vigorous seedling could develop these protective barriers, which would enable it to resist invasion more effectively than would the slower growing seedlings of the same variety. Partial correlations show that size of seed influenced the reaction of the seedlings to seedling blight in seed lots produced in 1930 but not in 1929, and that it had more influence on seed lots of barley than of wheat. In fact, a negative correlation was obtained between size of seed sown and resistance to seedling blight in Hard Federation. It seems probable, however, that the true association is not between small seed and resistance but rather between resistance and some condition associated with small seed. The nature of this condition is not apparent from the present studies. The fact that plants from seed harvested 11 days after flowering at Moccasin, Mont., in 2 successive years had the same reaction to seedling blight as plants from mature seed and the further fact that plants from Madison-grown seed, even though it was shriveled, were infected less than plants from plump, well-developed seed from other stations indicate that the stage of development of the embryo or endosperm in the seed is not of great importance in determining the reaction of wheat to seedling blight caused by *Gibberella saubinetii*.

In view of the fact that size of seed and vigor of seedling growth account for only part of the differences in reaction to seedling blight of seed lots of the same variety, it would seem that a host-parasite relationship of nutritional nature might be partially responsible. This is in line with the work of Dickson (*3, 4*), who has shown that the parasitic relation between *Gibberella saubinetii* and the seedlings of corn and wheat grown under different temperatures may be determined by differences in composition of the host plants. If host-parasite relationships can be altered so radically by a change in the composition of seedlings grown under different temperatures, it seems logical to expect these relationships to be altered as a result of differences in the chemical and physiological composition of the embryo and of the endosperm which furnishes food for its early growth.

The results of Dickson (*3*) show that wheat seedlings grown at low temperatures have relatively low nitrogen and high total sugar and dextrin content and are very resistant to seedling blight caused by *Gibberella saubinetii*. When grown at temperatures above 12° C. the nitrogen content increases and the total sugar and the dextrin content decrease and the seedlings are susceptible. The negative correlation found in the present studies between protein content of the seed lots and high indices of resistance to blight suggests a similar relation. While no data are available from the present study on the influence of chemical composition of the seed on composition of the resulting seedlings, it seems logical to expect that the balance between nitrogen and carbohydrates in the seed would be reflected in the germinating seedlings, and that seedlings from seeds relatively low in

nitrogen would be relatively high in carbohydrates, at least during the period when infection may occur.

The embryos in seeds of cereals (which are formed in the environment of the mother plant) contain the young plant with seminal roots, leaves, and the growing point of the axillary coleoptile tiller differentiated. Much evidence is available to show the variation that may occur in the chemical composition of the endosperm, which sustains the seedling during the first few days after germination, depending on the environment under which it was produced. The embryo in corn was shown by Hopkins et al. (*6*) to vary as widely in its chemical composition as the endosperm. Toole (*12*) has shown that the embryo starts growth before any changes take place in the endosperm, i. e., the embryo utilizes its own food reserve in starting growth. The seedling could, therefore, be in an active growing state before it was influenced by outside nutritional conditions even from the endosperm.

While the present studies are not conclusive, they do suggest that reaction to seedling blight of the seed lots of a variety grown under different environments is determined largely by a host-parasite relationship of a nutritional or biochemical nature rather than by differences in the morphological development of the embryo or size of the endosperm. Yield of grain and reaction to bunt may also be influenced by chemical and physiological composition as well as by size of seed sown, but the correlation between size of seed sown and these characters would seem to account for most of the differences observed in the present study.

The results indicate the importance of carefully selecting seed for conducting varietal tests for resistance, at least to some diseases. Differences in the source of the seed resulted in greater differences in reaction to seedling blight than were obtained between varieties. Similar results are more likely to be expected for characters that are expressed during the seedling stage than for characters expressed later in the life of the plant.

SUMMARY

Seed of four varieties of spring wheat and three of spring barley produced at several experiment stations in the United States and Canada in 1929 and 1930 was tested for reaction to seedling blight in the greenhouse at Madison, Wis., and for reaction to bunt and for yield in field trials at Moccasin, Mont., Madison, Wis., and Aberdeen, Idaho.

Environmental conditions under which the seed was produced affected the reaction to seedling blight (*Gibberella saubinetii*) of the subsequent crop. A relationship was found between low protein in the seed and high resistance indices and in some cases between large seed and high resistance indices, but these factors did not account for all differences. The results suggested that differences of a nutritional or biochemical nature were more important than morphological or size differences in the embryo and endosperm. Differences in disease reaction between seed of the same variety produced under different environmental conditions were greater than differences between varieties from seed produced at the same station. These results emphasize the necessity of carefully selecting seed for testing varietal resistance.

The environmental conditions under which the wheat seed was produced also affected the reaction to bunt, but the effects were much less pronounced than in the case of seedling blight. Only 1 of 76 seed

lots tested was considered to differ significantly from other seed lots of the same variety. However, a small correlation (−0.3036, $P=0.01$) was found between size of seed and reaction to bunt, the larger seed producing a lower percentage of infected plants.

Only 3 of the 91 individual seed lots produced under different environmental conditions differed significantly from other seed lots of the same variety in capacity to yield when a difference twice its standard error was considered as significant. Statistically significant correlation coefficients were obtained, however, for weight of 1,000 kernels sown and dry weight of 100 seedlings at about the fifth-leaf stage (+0.9312, $P<0.01$ for wheat and +0.8213, $P<0.01$ for barley) and for weight of 1,000 kernels sown and yield of grain (+0.4026, $P=0.02$ for wheat and +0.4049, $P=0.05$ for barley).

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VARIABILITY OF FIBER LENGTH IN A RELATIVELY UNIFORM STRAIN OF COTTON [1]

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INTRODUCTION

The general conception of a uniform sample of cotton (*Gossypium*) is one in which a large proportion of the fibers are the same length. With few exceptions, cotton breeders and other investigators who work with seed cotton have used the combing or butterfly [3] measurement of the fibers on a seed as an expression of lint length. The combing length is usually considered to be the modal length, although the actual mode can be determined only by some form of fiber sorting. The standard deviation of the combing lengths has been frequently used as a measure of the variability of the sample.

Continued selection of plants or progenies with low standard deviations of combing lengths may tend to reduce the total variation in lint length, but the investigator who is familiar with the natural diversity in the length of fibers on individual seeds will appreciate the limitations on progress toward uniformity by this method. This paper describes the methods used in determining the variability of length and gives the results of fiber-length studies of a strain of Pima cotton (*Gossypium barbadense* L.) grown at Sacaton, Ariz., in 1934.

TYPES OF COTTON FIBER SORTERS

A sorter for determining the length variability of ginned samples of cotton has been described by Webb (6).[4] Small pulls of paralleled fibers are drawn from the machine and laid out in the decreasing order of their lengths on boards covered with velvet. Each pull is measured and recorded in its proper length class. The class range is usually one-sixteenth or one-eighth of an inch, depending on the detail desired. The weight of the fiber is substituted for the frequency in calculating the variability of the sample, because of the impracticability of counting the individual fibers in each class.

Pressley (4) developed a machine for the sorting of cotton fibers from the seed. The combs in this sorter are one-eighth of an inch apart, and in addition to their function of holding the fibers in a parallel position, they give a measure of the fiber length. Another device for sorting fibers from the seed has recently been announced by McNamara and Stutts (3). The combs on this machine also are one-eighth of an inch apart. A sorter of this type was used in making the fiber arrays reported in this paper.

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[2] The writers are indebted to G. N. Collins, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for valuable advice concerning the application of the analysis of variance to their problem, and to E. Gordon Smith, of the Division of Cotton and Other Fiber Crops and Diseases, for aid in making the fiber combings and arrays.
[3] After the fibers on a seed have been straightened by combing, the distance from the point of attachment to the point where the fibers become noticeably scant may be called the combing length.
[4] Reference is made by number (italic) to Literature Cited, p. 762.

COMPARISON OF METHODS OF MEASUREMENT

Preliminary work on fiber arrays in which the several pulls were laid out on velvet-covered boards, measured, and then grouped and weighed has shown this to be a rather long and tedious procedure. According to the method reported by Pressley (*4*), all the fibers that occur between a given pair of combs are drawn out and weighed. Since the combs are spaced at regular intervals of one-eighth of an inch, they are used as the measure of the length of the fiber taken between each succeeding pair of combs. The method of actually measuring each pull with a ruler was compared with the method in which the combs of the McNamara sorter were used as the measure. By carefully laying out each pull and making proper notations of the pulls that occurred between each succeeding pair of combs in the sorter, both methods were compared on each fiber array. After each pull was measured, instead of weighing the combined pulls in a length class, as is the normal practice, each pull was weighed separately. One grouping of weights was made into the length classes as determined by the ruler and another grouping into the length classes as determined by the number of pulls between each sorter comb. Twenty single-seed samples were handled in this manner, and the mean length and variance of the fibers on each seed were calculated.

An average of the mean lengths showed that the comb method gave a slightly longer measurement than the ruler method, the mean difference, expressed as a fraction of a class length, being 0.1425 ± 0.0479, or 0.0178 inch. The probability of the occurrence of a fortuitous deviation as great or greater than the designated one is 27 in 10,000. However, the chances are 99 to 1 that the difference will be less than one sixty-fourth of an inch. While the odds seem rather high for the observed difference, the actual deviation is much smaller than could be detected by the method of measurement, and since neither method can be considered a standard it was assumed that either could be used as a measure of the length if both gave the same measure of variability of the fiber on the seed.

A comparison of the variance of the fiber on the seed, calculated from data obtained by both the ruler and comb methods, showed that a difference as large as one-seventieth of an inch would occur only once in 100 times. These findings were the basis for the adoption of the comb method of measurement for subsequent studies in fiber distribution.

CONSOLIDATION OF SUBSTAPLE LENGTH CLASSES

The proportion of fibers shorter than three-fourths of an inch in these samples was about 5 percent. Since this substaple is considered by spinners to be of doubtful value and information regarding its length distribution was not needed, it seemed practical to consolidate all of these shorter lengths into one class, thus reducing further the time and labor of arraying a sample of cotton. Using the same arrays described in the preceding section, the weights of all length classes under three-fourths of an inch were grouped together and treated as one class. When these arrays so grouped were compared with arrays of the same samples in which the fibers were placed in ⅛-inch groups ranging from the longest to the shortest, the procedure by which the length classes under three-fourths of an inch were combined gave a difference of mean lengths which was 0.0175 ± 0.0122 of a class length longer than these same arrays when the shorter lengths were not combined. The probability of a fortuitous occurrence of a deviation of this size is 16 in 100.

DIFFERENCES IN MEAN LENGTH OF FIBERS FROM RIGHT AND LEFT SIDES OF SEED

In the preparation of the seed-cotton samples for sorting, the method outlined by Cook (*1*) for parting the fibers on the raphe or seam of the seed was followed (figs. 1 and 2). The same methods of preparation and sorting were applied to each seed. When the seed on which the fiber had been parted was placed with the raphe side up and the tip or chalazal end pointed toward the operator, the side of the seed on the operator's right was designated as the right side and the other side was designated as the left. It was possible, by a comparison of the mean lengths (as determined from the fiber-array analysis) of the fiber from the right side of a seed with that from the left side, to determine the differences in fiber length between the two sides of the seed. Using the method of sorting previously described, the fibers from the right and left sides of seeds from four samples per plant from 19 plants were arrayed. The mean lengths of the fibers from the right side of the seed averaged 0.6919 ± 0.1023 of a class length, or 0.0865 inch longer than the means of the left side. The significance of this difference is emphasized by calculating the maximum difference that could be expected to occur by chance once in 100 times, which in this case was found to be 0.033 inch.

Whether this difference in mean length of the fibers on the right and the left side of the seed is inherent or is due to the technique of the operator was not determined. However, the fact is that measurable differences were observed when methods that seemed most practical for this work were used, and in view of these findings it seems that the proper expression of the mean length of the fibers on a seed may be obtained only by sorting the fibers combed out on both sides of the seed. Because of this necessity it was impossible to get two strictly comparable measures of length from a single seed and thus calculate the experimental error of the method of determination of the distribution of the fibers on a single seed.

CALCULATION OF MEAN LENGTH AND STANDARD DEVIATION OF FIBERS FROM INDIVIDUAL SEEDS

The calculation of the mean length, standard deviation, etc., of an array of fibers from a cottonseed used in this experiment followed the familiar method where the assumed mean, 0, is used in determining the squared departures. The length classes were expressed in $\frac{1}{8}$-inch units; the weight of the fibers in each class (to the nearest 0.02 mg) was substituted for the number of fibers as the frequency.

When l=length of a class
$\quad$ w=weight of fibers in a class
$\quad$ $W = \Sigma w$

the various formulae are as follows:

$$\text{Mean} = \frac{\Sigma lw}{W}$$

$$\sigma^2 = \frac{\Sigma l^2 w - \dfrac{(\Sigma lw)^2}{W}}{W-1}$$
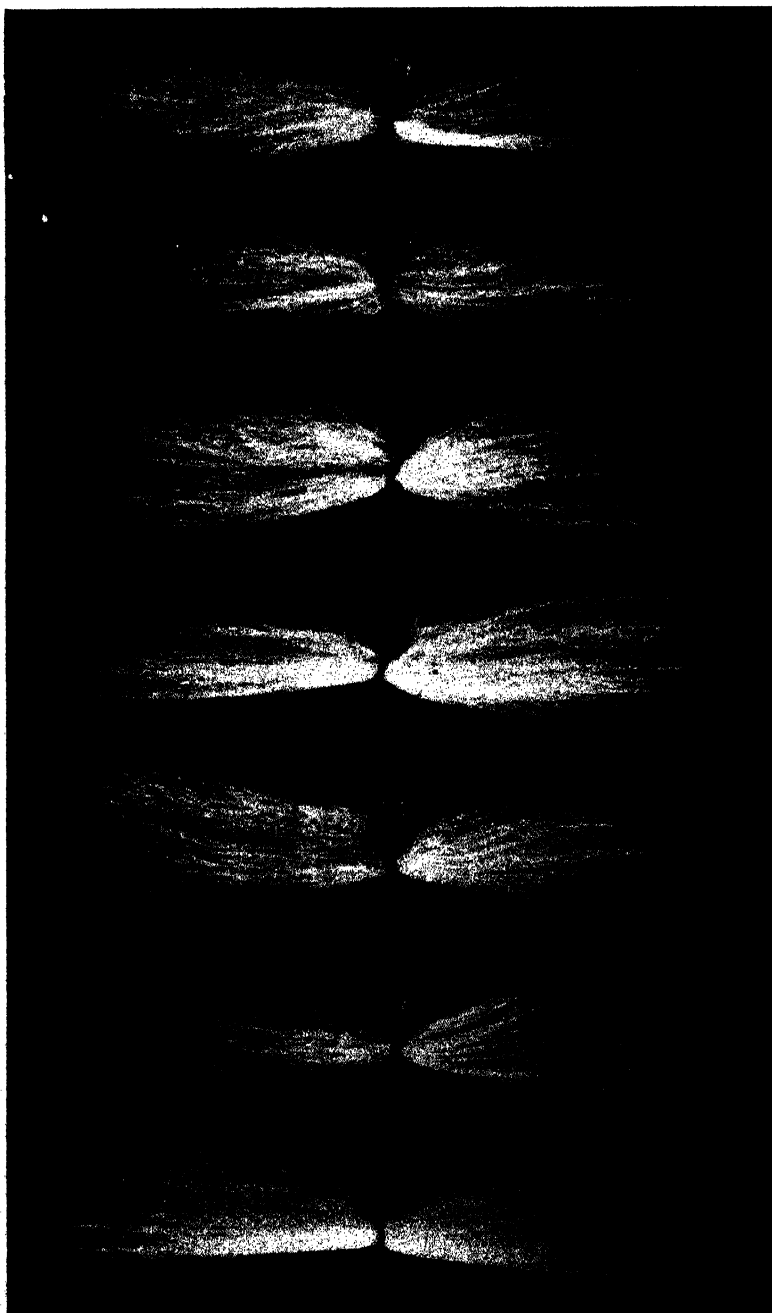
FIGURE 1.—Combings from random samples from consecutive Pima cotton plants, showing relative uniformity of the combing lengths. Natural size.
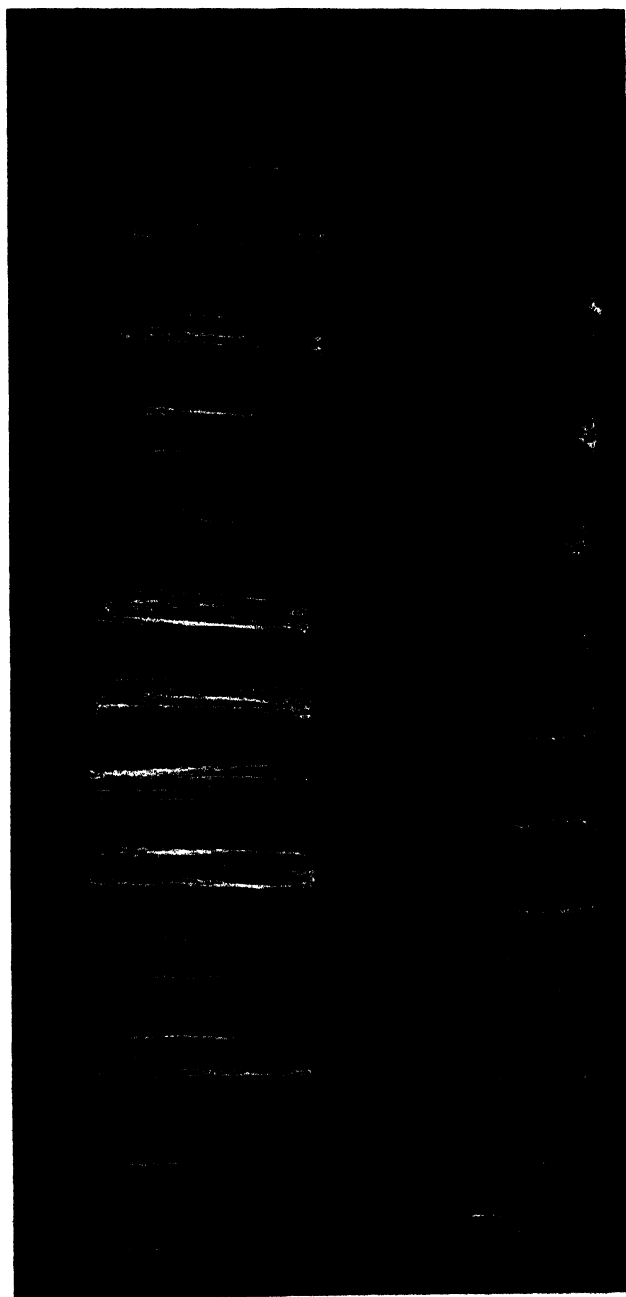
FIGURE 2.—Fiber array, showing the variation in length of fibers from a single seed.   Three-quarters natural size.

The data secured from the fourth sample from Pima plant no. 4 follow:

l	w
14	0. 58
13	2. 78
12	6. 86
11	7. 38
10	4. 86
9	3. 94
8	3. 24
7	2. 28
6	1. 38
4 [1]	3. 80
	37. 10

[1] Classes 5 to 1 (less than ¾ inch) are consolidated.

When the formulae just given are applied to these data, the results are as follows:

$$\frac{\Sigma lw}{W}=\frac{357.18}{37.10}=9.627\ (=1.204\ in.)=M.$$

$$\Sigma l^2 w=3,699.020$$
$$\frac{(\Sigma lw)^2}{W}=3,438.748$$
$$\overline{260.272}$$

$$\frac{260.272}{(37.10-1)}=7.2098=\sigma^2$$

$$2.6851=\sigma$$

VARIATION IN FIBER LENGTH DUE TO POSITION IN THE LOCK

A lock of cotton is composed of several seeds held together by the natural entwining and intermingling of the fibers. Extreme care must be exercised if a seed is to be disengaged without detaching a considerable amount of fiber. It was found to be impracticable to separate the seeds of a lock in such a way that individual seeds could be taken at random. Therefore, the lock, which is the natural unit in seed cotton, was taken at random. In order to prevent discrimination in sampling, it was necessary to determine the amount of variation in mean length of the fibers on individual seeds within the lock that was due to the position of the seed in the lock. Two locks were taken at random from the bulked cotton from each of 19 consecutive plants. A seed from the bottom, middle, and top positions was taken from each lock, and fiber arrays were made of each. The mean length of the fibers on each seed was determined, an analysis of the variance of which is given in table 1. It will be noted that F, as derived by the method outlined by Snedecor (5) for means of positions, is well under the F value for a probability of 0.01.[5] From the standard error as calculated from the remainder variance, the chances are found to be 99 to 1 that the difference in mean lengths of the fibers among the three positions is less than one thirty-second of an inch. The F value found in the analysis in table 1 indicates that the samples from the three positions in the lock used in this study may be considered

[5] The significance of F, which is an expression of the relation of the larger mean square to the smaller, is influenced by the number of degrees of freedom in the two mean squares. Tables have been computed which give the F values, for appropriate degrees of freedom, for probabilities of 0.05 and 0.01.

homogeneous in mean fiber lengths, and this would ordinarily preclude further comparisons in which the error of the difference of part of the data was used (7).

TABLE 1.—*Analysis of variance of mean lengths of fibers on individual seeds from top, middle, and bottom positions in each of two locks per plant from 19 plants of Pima cotton*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	113	51.4997	0.4557
Within classes [1]	57	25.7921	.4525
Means of plants	18	21.6540	1.2030
Means of positions [2]	2	.6851	.3426
Remainder	36	3.3085	.0936

[1] This portion of the analysis is the variance of lock positions on single plants, and is not to be confused with "means of positions", which is the variance among positions from all plants.

[2] For means of positions, $F = \frac{0.3426}{0.0936} = 3.66$. For a probability of 0.01, F would be 5.27.

The following data show comparisons of the average of the mean fiber lengths on seeds from top, middle, and bottom positions in locks from two locks from each of 19 plants.

Position in locks   Fiber length (M±S. E.)

Middle	class lengths [1]	9.815 ± 0.0988
Bottom	do	9.631 ± .1218
Difference	do	.184 ± .1568
Do	inch	.023 ± .0196
D/E	ratio	1.17

Middle	class lengths	9.815 ± .0988
Top	do	9.733 ± .1076
Difference	do	.082 ± .1461
Do	inch	.010 ± .0183
D/E	ratio	0.56

Top	class lengths	9.733 ± .1076
Bottom	do	9.631 ± .1218
Difference	do	.102 ± .1625
Do	inch	.013 ± .0203
D/E	ratio	0.63

[1] One class length = one-eighth of an inch.

Since the mean lengths of fibers from seeds within the three positions measured did not differ significantly, it was believed that a seed taken from any part of the lock would be representative, but as a precaution against the introduction of an error due to some position not being accounted for in this analysis, a seed from the middle of each lock (the lock having been taken at random) was used as the sample in subsequent studies.

RELATION OF THE MEAN TO OTHER METHODS OF MEASUREMENT

In this study the mean is used as the expression of the length of fibers on a seed. It is calculated in the usual manner by multiplying each class length by the weight of the fibers in that class and dividing the sum of lengths × weights by the sum of the weights. In addition

to the mean, Webb (*6*), in his work with the ginned lint, calculated the length at the upper quartile. This is the length of the group at that point on the array which includes 75 percent by weight of the fibers, beginning with the shortest groups. From the data given in the example on page 754, the upper quartile length is calculated as follows:

Find 25 percent of the total weight of the sample:

$$37.10 \text{ mg} \times 0.25 = 9.275 \text{ mg}$$

Find the number of classes, beginning with the longest, the sums of the weights in which will come nearest to equaling $\Sigma w \times 0.25$ but will not exceed it. Classes 14 and 13 may be summed, but the addition of class 12 brings the total above 9.275 mg.

From 9.275 subtract 3.36 (Σ classes 14 and 13), leaving 5.915 mg, which is the amount of the upper quartile extending into class 12. Since class 12 contains 6.86 mg, the percentage of class 12 in the upper quartile may be calculated by dividing 5.915 by 6.86, which gives 0.8622. Subtract this factor from the length of the next higher class: $13 - 0.86 = 12.14$ (eighths of an inch), or 1.52 inches.

The combing length is the measurement in general use by cotton breeders. Although the method to be used for measuring may depend on the preference of the investigator, the usual method of preparing the sample is that reported in this paper. A system of grades together with an easy and convenient method of measuring the combing length of the cotton lint while attached to the seed has been developed by Kearney (*2*), and this method was used in determining the combing length of the samples used in this study. The average of the 76 determinations of mean fiber length was 9.872 class lengths of one-eighth of an inch each ($=1.234$ inches); the average of the upper quartile lengths was 1.519 inches; and the average combing length was 7.71 grades ($=1.589$ inches).

The correlation of the mean length with the upper quartile length and with the combing length, as determined from 4 samples from each of 19 consecutive plants, is given in table 2. Reference to this table shows that the correlation coefficient for the mean with combing-length measurement is $+0.8049$ and that for the mean with upper quartile length is $+0.9518$. In view of the very close relationship between these expressions of lint length, it is apparent that after one has been determined the others may be predicted with a considerable degree of accuracy.

TABLE 2.—*Analysis of variance and covariance of different methods of measuring fiber length, as determined on the fiber of 4 seeds from each of 19 plants of Pima cotton*

MEAN LENGTH AND COMBING LENGTH

Source of variation	Degrees of freedom	Mean lengths		Lengths		Covariance	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of products	Coefficient of correlation
Total	75	25.4175	0.3389	29.1316	0.3884	21.9010	$+0.8049 \pm 0.0404$
Means of plants	18	8.6328	.4796	10.0066	.5559	7.6970	$+.8281 \pm .0721$
Samples on plant	57	16.7847	.2945	19.1250	.3355	14.2040	$+.7928 \pm .0488$

MEAN LENGTH AND UPPER QUARTILE LENGTH

Total	75	25.4175	0.3389	0.5174	0.0069	3.4515	$+0.9518 \pm 0.0108$
Means of plants	18	8.6328	.4796	.1739	.0097	1.1842	$+.9665 \pm .0151$
Samples on plant	57	16.7847	.2945	.3435	.0060	2.2673	$+.9443 \pm .0142$

SOURCES OF VARIATION IN FIBER LENGTH

The development of a practical method of sorting lint from the seed offers opportunities for further investigation of the sources of the variation in fiber length. Four random samples (locks) were taken from each of 19 consecutive plants in a row of a commercial strain of Pima cotton growing in the yield test at Sacaton, Ariz., in 1934. In accordance with the procedure adopted, a seed from near the middle of each lock was taken, combings were made of each seed, and the combing length was graded. The fibers from each seed sample were sorted, and the mean length, the upper quartile length, and the variance were calculated. The total variance in mean lengths was apportioned between that which was due to variation among the means of different samples from the same plant and that which was due to variation among the means of plants in the row, as shown in table 3. The mean squares of the two sources of variation are so nearly equal that the samples must be considered as coming from a homogeneous population. The F value for means of plants is well below the F value for a probability of 0.01.

TABLE 3.—*Analysis of variance of mean lengths of fibers on individual seeds from 4 samples from each of 19 plants of Pima cotton*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	75	24.4173	0.3256
Means of samples from the same plant	57	15.7846	.2769
Means of plants [1]	18	8.6327	.4796

[1] For means of plants, $F = \left(\frac{0.4796}{0.2769}\right) = 1.73$. For a probability of 0.01, F would be 2.12.

An adaptation of the method of analysis of variance which expresses the extent to which variation of the lint on the seed contributes to the total variance of the composite sample has been suggested by G. N. Collins. The formulae and an example of this method, involving 2 samples per plant from the first 10 plants used in this experiment, follow.

To subdivide the variation in a composite sample into the portions contributed by (1) departures in the length of fibers from the mean of the seed on which they are borne; (2) departures in the mean lengths of fibers on the seeds from the mean of the plant; and (3) departures in the mean length of fibers on the individual plants from the general mean,

Let: W_s=weight of fibers on a seed,
W_p=weight of fibers on a plant,
W_t=total weight of fibers,
lW_s=sum of the products (length×weight) on a seed,
lW_p=sum of the products (length×weight) on a plant,
lW_t=sum of the products (length×weight) for entire population,
l^2W_s=sum of the products (squared length×weight) on a seed.
Then:
Sum of squared departures on the seed=

$$l^2W_s - \sum \frac{(lW_s)^2}{W_s} \tag{1}$$

Sum of squared departures among means of sample on the same plant=

$$\sum \frac{(lW_s)^2}{W_s} - \sum \frac{(lW_p)^2}{W_p} \hspace{3cm} (2)$$

Sum of squared departures among means of plants=

$$\sum \frac{(lW_p)^2}{W_p} - \frac{(lW_t)^2}{W_t} \hspace{3cm} (3)$$

Table 4 shows data for fiber lengths on 2 seeds from each of 10 plants. By means of the formulae just given, the analysis of variance in table 5 was calculated from the data in table 4.

TABLE 4.—*Data from sorting of fibers from 2 seeds from each of 10 plants of Pima cotton*

Plant no.	Sample no.	W_s	W_p	lW_s	lW_p	$l^2 W_s$	$\frac{(lW_s)^2}{W_s}$	$\frac{(lW_p)^2}{W_p}$
1	2	3	4	5	6	7	8	9
1	1	43.84	81.00	424.16	810.84	4,289.44	4,103.8254	8,116.8087
	2	37.16		386.68		4,242.32	4,023.7196	
2	1	33.20	71.66	336.06	692.82	3,563.22	3,401.6064	6,698.2913
	2	38.46		356.76		3,549.60	3,309.3525	
3	1	42.68	79.90	428.92	804.24	4,539.48	4,310.5053	8,095.1436
	2	37.22		375.32		3,996.76	3,784.6615	
4	1	39.80	79.44	382.42	791.10	3,926.38	3,674.4989	7,878.1371
	2	39.64		408.68		4,450.36	4,213.4041	
5	1	36.78	81.14	352.04	824.12	3,554.92	3,369.5530	8,370.3941
	2	44.36		472.08		5,248.56	5,023.8847	
6	1	35.02	69.68	325.52	660.24	3,201.16	3,025.7929	6,255.9825
	2	34.66		334.72		3,434.12	3,232.4719	
7	1	41.40	81.76	400.78	762.48	4,106.26	3,879.8214	7,110.7602
	2	40.36		361.70		3,417.70	3,241.4987	
8	1	35.38	79.02	377.96	829.06	4,251.56	4,037.6981	8,698.3103
	2	43.64		451.10		4,926.14	4,662.9516	
9	1	44.20	81.46	452.82	804.30	4,953.94	4,639.0486	7,941.3024
	2	37.26		351.48		3,527.20	3,315.5714	
10	1	44.78	74.78	415.74	725.70	4,097.06	3,859.7531	7,042.5313
	2	30.00		309.96		3,395.40	3,202.5067	
Total		779.84	779.84	7,704.90	7,704.90	80,671.58	76,312.1258	76,207.6615

Sum of squares on the seed, formula (1).—In table 4 the total of column 7=$\sum l^2 W_s$=80,671.58, and the total of column 8=$\sum \frac{(lW_s)^2}{W_s}$=76,312.1258. The difference, 4,359.4542, is the sum of squares on the seed.

Sum of squares among means of samples on the plant, formula (2).—The first term of formula (2), $\sum \frac{(lW_s)^2}{W_s}$, is the total of column 8, and is 76,312.1258. To calculate the second term, the values in column 6 are squared and divided by the corresponding values in column 4. The sum of these quotients=$\sum \frac{(lW_p)^2}{W_p}$=76,207.6615. The first term minus the second term, 76,312.1258−76,207.6615=104.4643, is the sum of squares among means of samples on the same plant.

Sum of squares among means of plants, formula (3).—The first term, $\sum \frac{(lW_p)^2}{W_p}$, as just calculated, is 76,207.6615. The second term, $\frac{(lW_t)^2}{W_t}$, is the total of column 5, squared and divided by the total of column 3, and is 76,125.2103. The first term minus the second, 76,207.6615−76,125.2103=82.4512, is the sum of squares among means of plants.

Total sum of squares.—It will be noted that the correction term in each formula becomes the first term in the succeeding formula.   Thus the first term in formula (1) minus the correction term in formula (3), 80,671.58 − 76,125.2103 = 4,546.3697 = the sum of three variances and is the total variance.

In calculating the degrees of freedom the grand total of the weights used as the frequencies is rounded off to the nearest milligram for the final divisions in table 5 (sum of squares÷degrees of freedom = mean square).   Thus for the total there were 780 − 1, or 779; on the seeds, 1 degree of freedom on each of the 20 arrays was sacrificed, leaving 780 − 20, or 760; among means of samples from the same plant there were 2 samples per plant with 1 degree of freedom per plant, or 10 for the 10 plants; and among the means of plants there were 10 − 1, or 9.

The analysis of variance of the 76 seed samples previously discussed is given in table 5.   This table shows substantial agreement with the length measurements which were taken on mean lengths alone, as given in table 4.   There was no significant difference in the mean square determinations between two samples from each of 10 plants (table 5, column 4) and four samples from each of 19 plants (table 5, column 7).   The variation among means of plants is no greater than might be expected from the variability of samples from the same plant.   Compared with the variation of fibers on the seed, however, the variability of plant means appears significant.   The latter comparison indicates that differences among means of plants were detected and that this variance, although an insignificant part of the total, was larger than was expected from a random sample having the variance of the fibers on individual seeds.   Even with complete genetic uniformity, variations in plant means are to be expected as a result of variations in environment.   In the present instance the differences in plant means undoubtedly would have been greater had the plants been selected from scattered areas in the field.

TABLE 5.—*Analysis of variance of fiber length in composite samples of fiber from plants of Pima cotton*

Source of variation	Fibers from 2 seeds from each of 10 plants			Fibers from 4 seeds from each of 19 plants		
	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square
Total	779	4,546.3697	5.8362	2,779	17,516.1269	6.3030
On seeds	760	4,359.4542	5.7361	2,704	16,673.1265	6.1661
Means of samples from the same plant	10	104.4643	10.4464	57	551.1960	9.6701
Means of plants	9	82.4512	9.1612	18	291.8044	16.2114

Comparison of mean squares	Fibers from 2 seeds from each of 10 plants		Fibers from 4 seeds from each of 19 plants	
	Value of F	Value of F for $P=0.01$	Value of F	Value of F for $P=0.01$
$\dfrac{\text{Means of samples from the same plant}}{\text{On seeds}}$	1.82	2.25	1.57	1.79−
$\dfrac{\text{Means of samples from the same plant (or reciprocal)}}{\text{Means of plants}}$	1.14	4.31	1.68	2.50
$\dfrac{\text{Means of plants}}{\text{On seeds}}$	1.59	2.53	2.63	1.80

The standard deviation of fibers on the seed (2.4802) is only 1.1 percent less than the observed standard deviation of the entire composite sample (2.5106). This may be taken to indicate that if there were no differences in the true means of fiber length among samples from the same plant or among means of plants the variation of the composite sample would be reduced 1.1 percent. In other words, these data show that in this sample of Pima cotton more than 98 percent of the variability in lint length as measured by the standard deviation was due to the variation of the fibers on the seed. The amount of variation contributed by the fibers on the seed is further emphasized in figures 1 and 2. The combings shown in figure 1 were made from random samples from consecutive plants; figure 2 shows a fiber array made from a representative seed. It is obvious that the
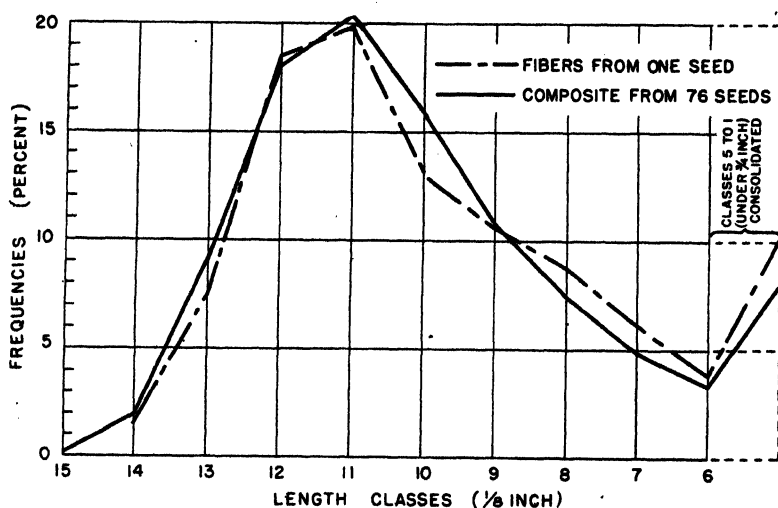
FIGURE 3.—Comparison of the frequency distribution of lengths of the fibers on a single representative seed of Pima cotton with that of a composite sample of the fibers from four seeds from each of 19 Pima plants.

variation in combing lengths in figure 1 is very much less than that of the fibers from the individual seed as shown in figure 2. The same idea is brought out graphically in figure 3. The solid line represents a composite array of 76 individual seed samples, and the broken line represents the distribution of fiber lengths on a single seed. Although in both graphs the class lengths are exaggerated, the cumulative curves in figure 4 show, even more strikingly than the curves in figure 3, the similarity between the frequency distributions of the fibers from 1 seed and from 76 seeds, respectively.

DISCUSSION AND CONCLUSIONS

The fiber-sorting methods developed in this study were incidental to the analysis of variance of the fiber lengths in a variety of cotton. In Pima cotton, the variety used in this study, the general plant characters have been rather definitely fixed through many years of inbreeding followed by careful roguing each year after the seed distribution reached commercial proportions. Since the samples were

obtained from Pima cotton plants that were normal in appearance, having been cultivated and irrigated in accordance with accepted practices, and from a small section of a row in which the plants were uniform in growth, it was not surprising that there was little variation either among the means of different samples from a single plant or among the means of plants in the row. The results obtained in this study show that the variation in fiber length on seeds was responsible for as much as 98 percent of the total variation in the composite sample and indicate that if further reduction in the total variance of the fiber lengths in composite samples of Pima cotton is desired the most rapid approach would be by breeding for a reduction of the variance of the fiber lengths on seeds.

The general plant and fiber characters in some varieties may not be as well fixed as in Pima. Combing length measurements of seed samples of cotton from mixed or deteriorated seed stocks have often given a wide range of differences. Obviously, the total variance in
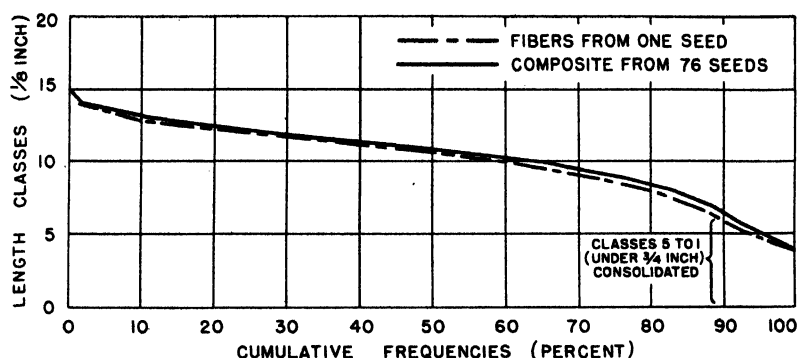
FIGURE 4.—Ogive curves comparing an array of fibers from a single representative seed of Pima cotton with that of a composite sample of the fibers from 4 seeds from each of 19 Pima plants.

fiber length among consecutive plants grown from such a seed stock would be geater than that which was found in Pima cotton, but even in extremely diverse stocks the variability of the fibers on individual seeds would contribute a large proportion of the total variance.

The extent or intimacy of the mixture of the fibers from different seeds, which make up the ginned sample, has not been determined, but it is believed that the mixture is thorough enough to be considered a representative sample of the fibers from the individual seeds. When composite or ginned samples are used, variations in the fiber length contributed by individual plants cannot be detected and plants with wide differences in mean lengths of fiber may make a composite fiber array, the mean length of which would be little different from that of a uniform variety. The dispersion of the fibers undoubtedly would be greater in the mixed sample, as would be the usual case where cottons become mixed under commercial conditions. Sorting of seed-cotton samples affords a method by which the variation of fibers on seeds can be studied, and the application of the analysis of variance makes it possible to express this variation as its proportionate part of the total.

The cotton breeder has been confused by the broad usage of the term "fiber-length uniformity." Two schools of thought, each with numerous variations, may be recognized. One, which is supported by

the majority of spinners, cotton technologists, and cotton breeders, thinks of uniform cotton as a sample in which all of the fibers tend to approach one length. The other group, which seems to be in the minority at present, thinks of a uniform sample of cotton as one made up of fibers from seeds which, if combed and sorted while attached to the seeds, would have had similar means, modes, and standard deviations. No attempt has been made in this study to determine which kind of uniformity is desired by the spinners, but from the standpoint of the cotton breeder there is an urgent need for a decision on this question. If the first idea proves to be correct, plant selection in well-bred varieties to increase the uniformity of mean fiber length hardly can be expected to produce any reasonable increase in the fiber uniformity of the composite or ginned sample. Considerable improvement in fiber uniformity of this type may be expected by selecting plants with low standard deviations of lint length as determined from fiber arrays of seed cotton. If the other kind of uniformity is desired, selection on the basis of plant means would, of course, be the proper procedure; but it would seem that thorough mixing of the fibers of stocks that vary somewhat in plant means would be equally advantageous. The second conception of fiber uniformity would obviate the necessity of sorting the fiber on individual seeds and calculating its variability.

SUMMARY

With samples from a commercial strain of Pima cotton, fiber-sorting methods were developed which materially reduced the time required for making fiber arrays of cottons of the Egyptian type.

The mean and the upper quartile length, which were determined from fiber arrays from the same seeds, were found to be highly correlated, as were the mean and the combing length.

The differences from sample to sample on the plant and from plant to plant in the row were insignificant, and analysis of variance showed that the samples could be considered as coming from a homogeneous population.

Of the total variance in fiber length, as determined from sorting the fibers on the seed, the variance on the individual seeds contributed 98 percent, leaving the remainder attributable to that which occurred among the means of samples on the plant and among the means of plants in the row.

The recognition of the amount of variance that is due to the variation in the fiber length on the seed raises questions as to the definition of the term "uniform fiber length" and the methods to be followed by the cotton breeder in cotton improvement work.

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NITROGEN UTILIZATION BY OPHIOBOLUS GRAMINIS [1]

By HURLEY FELLOWS

ssociate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

It is an established fact that there exists a specificity among microorganisms for the kind of nitrogen compound they will utilize in their metabolism. Some are able to utilize many kinds of these compounds, others only a few.

Most of the nutritional work on fungi has been done with saprophytes. It seems that more should be done with the parasites. A knowledge of the nutritional requirements of parasitic fungi should be of aid in interpreting the phenomena of their specificity for certain hosts and of the method of hyphal penetration into plant tissue as well as the establishment of the organism after penetration. The differences in virulence among different species as well as among strains of one organism might well depend on the ease with which the available food can be utilized.

The investigations herein reported were undertaken to determine to what extent *Ophiobolus graminis* Sacc., which causes take-all of wheat, is specific in its utilization of nitrogen from different sources. A brief summary of the results has been published elsewhere,[2] and it is the purpose of the present paper to give the complete report.

METHODS

Throughout this work a liquid modified Czapek's medium of the following formula was used:

Magnesium sulphate (MgSO₄)	grams	0. 5
Potassium dihydrogen phosphate (KH₂PO₄)	do	1. 0
Ferrous sulphate (FeSO₄)	do	. 01
Sucrose or other carbohydrate	do	30. 0
Nitrogen	Source and amount varied
Water	cubic centimeters	1, 000

The hydrogen-ion concentration of the medium was adjusted as nearly as possible to within a favorable range for the growth of *Ophiobolus graminis*. Pyrex flasks of 125-cc capacity were used as the receptacles for the medium. Fifty cubic centimeters of the prepared medium was introduced into these flasks.

Prepared media were steam sterilized at 15 pounds' pressure for 20 minutes, except when the media contained compounds known to break down at high temperatures. In such cases sterilization was intermittent in the steam sterilizer.

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Ophiobolus graminis was grown on potato-dextrose agar in Petri dishes. The agar with the adhering mycelium was cut into small blocks and one block was placed in each flask. The temperature of the growing cultures was maintained at 24° C. in an electric incubator. For the most part, growth observations were made by the unaided eye, and the relative abundance of growth was rated from none to the best by the symbols 0, 1, 2, 3, 4, and 5. In a few instances the mycelium was dried and weighed.

The nitrogen sources used consisted of both inorganic and organic compounds, as nearly chemically pure as can be purchased on the market. The organic nitrogen compounds included proteins of various complexity, representative members of the aliphatic and aromatic nitrogen series, the alkaloids, and miscellaneous compounds.

Certain nitrogen compounds probably are toxic to all forms of cryptogams. A pinch of garden soil was added to one flask of each type of culture to find whether any of the many organisms present would grow. Lack 'of all growth should be an indication of general toxicity. *Rhizopus* sp. and *Penicillium* sp., two vigorous growth types, were sown in other flasks for the same purpose.

It was necessary to exercise caution concerning the concentration of certain compounds that were being tested for the first time in a nutrient solution. A concentration that would permit growth was attained by adding to the culture solution containing the untried source of nitrogen a small amount of casein, which is utilized by *Ophiobolus graminis*. Growth under these conditions was taken to indicate that the material was not toxic, or if so, that the concentration was not great enough to be injurious. Two grams of any of the compounds tried in 1,000 cc of the medium was found not to be too concentrated.

RESULTS

NITROGEN COMPOUNDS AS NUTRIENT SOURCES

Table 1 shows the ability of *Ophiobolus graminis*, *Rhizopus* sp., and *Penicillium* sp. and of organisms in garden soil to grow in cultures in which nitrogen was supplied from various sources. Of all compounds tried, egg albumen, casein, peptone, and nucleic acid were the only ones that supported a growth of *O. graminis*, although many of them supported at least some growth of *Rhizopus* and *Penicillium*. Acetanilide, diphenylamine, cyanamide, dicyandiamide, and benzylamine appeared to be toxic to all the forms of life that were introduced. Hippuric acid, benzenesulphonamid, and betaine hydrochloride were evidently poor sources of nitrogen for all of the many fungi and bacteria from the soil.

Since *Ophiobolus graminis* utilized egg albumen and casein, it appeared possible that it might utilize some of the hydrolytic products of these proteins. Six of seven of such hydrolytic products of casein and five of the seven from egg albumen were tried without success. These products were included in table 1. Growth was also negative when these products were mixed in the same proportion that they are found to be present by analysis in the respective proteins.

TABLE 1.—*Relative growth of Ophiobolus graminis, Rhizopus sp., Penicillium sp., and fungi and bacteria from garden soil in Czapek's solution with nitrogen from different sources*

Source of nitrogen	Tests	Relative growth [1] of—			
		Ophiobolus graminis	Rhizopus sp.	Penicillium sp.	Soil organisms
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Ammonium carbonate	8	0	1	3	5 F.
Ammonium chloride	6	0	5	4	5 F.
Ammonium formate	2	0	3	4	5 F.
Ammonium nitrate	6	0	5	4	5 F.
Ammonium oxalate	6	0	1	5	5 F.
Ammonium phosphate, mono	7	0	5	4	5 B F.
Ammonium phosphomolybdate	6	0	5		
Ammonium sulphate	9	0	5	4	5 F.
Ammonium tartrate	4	0	5	4	5 F.
Calcium nitrate	2	0	0	5	5 B.
Magnesium nitrate	2	0	0	4	5 F.
Potassium nitrate	6	0			5 B F.
Sodium nitrate	5	0	5	5	5 F.
Sodium nitrite	2	0			5 F.
Organic:					
Acetamide	2	0	3	3	5 F.
Acetanilide	3	0			0.
d-alanine	2	0	5	2	5 F.
Albumen, egg	6	5			
Allantoin	2	0	5	2	5 B.
Asparagine	2	0	5	3	5 B.
Aspartic acid	2	0	5	3	5 B.
Benzenesulphonamid	2	0	0	0	T F.
Benzylamine	2	0	0	0	0.
Betaine hydrochloride	2	0	0	0	1 B F.
Caffeine	5	0	1		
Casein	Many	4			
Choline hydrochloride	2	0	2	3	5 B F.
Creatine hydrate	2	0	0	2	5 B.
Cyanamide	4	0			0.
Dicyandiamide	2	0	0	0	0.
Dinitrobenzoic acid	2	0	0	0	T F.
Diphenylamine	4	0			0.
Gelatin	2	0	3	2	3 B.
Glutamic acid	2	0	5	5	5 B.
Glycine	7	0	5	3	5 B.
Guanidine carbonate	2	0	0	4	5 B.
Hippuric acid	4	0			1
Hydroxylamine hydrochloride	2	0			3 F.
Leucine	2	0	5	2	5 F B.
Methylamine hydrochloride	2	0	2	2	5 B.
Nicotine	4	0	0		5 F.
Nucleic acid	2	2			
Peptone	5	5			
Phenylalanine	2	0	4	3	5 F.
Taurine	2	0	2	3	5 F B.
l-tyrosine	2	0	2	2	5 F.
Urea	2		50	4	5 F B.
Uric acid	4				5 F.
Control (no nitrogen)	Many	0	T		T.

[1] 0 indicates no growth, while 1 to 5 indicate 5 degrees of growth. F stands for fungi, B for bacteria, and T for trace.

EFFECT OF SOURCES OF CARBON

Dextrose, sucrose, maltose, starch, dextrin, lactose, *d*-fructose, and *l*-xylose were added separately to portions of several batches of media, each batch containing a different source of nitrogen. The nitrogen compounds were egg albumen, casein, peptone, sodium nitrate, ammonium chloride, ammonium phosphate, potassium nitrate, and calcium nitrate. *Ophiobolus graminis* grew well in all the media containing egg albumen, casein, and peptone, but not in the media containing other nitrogenous sources irrespective of the source of carbon. Weight measurements of the growth of *O. graminis* were made with dextrose, sucrose, maltose, starch, dextrin, and lactose when used in

combination with egg albumen, casein, and peptone. In all cases casein was the poorest source of nitrogen and peptone the best. It is not possible to tell from the data at hand which is the most favorable source of carbon, since *O. graminis* is able to utilize egg albumen, casein, and peptone themselves as a source of carbon. Growth is abundant in the presence of these three proteins when no other source of carbon is present. However, growth is better in all of these when a carbohydrate is present in the medium.

EFFECT OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

Additional experiments were conducted to determine whether the failure of certain compounds to support growth of *Ophiobolus graminis* might not be due to an unfavorable hydrogen-ion concentration. The compounds selected for study were ammonium nitrate, ammonium sulphate, ammonium carbonate, and ammonium chloride. Four batches of media were prepared, each containing a different one of these as a source of nitrogen. Each portion was subdivided into four parts and each part adjusted to a different hydrogen-ion concentration. The range was from pH 4.8 to 8.4. The growth in these different media was in no case greater than the neutral check, which had no nitrogen.

EFFECT OF GROWTH-PROMOTING MATERIALS

Separate batches of media containing the nitrogenous compounds potassium nitrate, ammonium sulphate, ammonium dihydrogen phosphate, ammonium oxalate, and glycine were each subdivided into five parts. A trace of a compound of copper, sodium, silicon, zinc, and aluminum was added separately to each subdivision. In no case did the growth of *Ophiobolus graminis* in these cultures differ from that of the checks containing the several nitrogen compounds without the addition of these elements.

Brenner [3] mentioned that traces of ammonium salts have been found to aid in the assimilation of the alkaloids by *Aspergillus niger* and *Penicillium glaucum*. Traces of ammonium chloride, ammonium tartrate, ammonium formate, and ammonium phosphate were therefore added separately to different samples of media containing caffeine and nicotine. In no case was the growth of *Ophiobolus graminis* improved. Both caffeine and nicotine as a source of nitrogen in Czapek's solution will support certain soil fungi and bacteria, but not *O. graminis*.

Mockeridge [4] did considerable work with accessory factors or growth-promoting substances for green plants. He found that water-soluble material from leafmold, well-rotted manures, fertile manured soils, and well-decayed peat greatly promote the growth of *Lemna major* in nutrient solution. This was not due solely to nutritive action. The writer tried a similar type of experiment with *Ophiobolus graminis*, using a few cubic centimeters of water extracts from garden soil, leafmold, stable manure, chicken manure, and peat to 50 cc of the medium containing the nitrogen compound to be tested. The source of nitrogen in the medium was ammonium chloride. The media were steam sterilized and the organisms were then added. Any

[3] Brenner, W. die stickstoffnahrung der schimmelpilze. Centbl. Bakt. [etc.] (II) 40: 555-647 illus. 1914.
[4] Mockeridge, F. A. the occurrence and nature of the plant growth-promoting substances in various organic manurial composts. Biochem. Jour. 14: [432]-450. 1920.

growth that occurred could be accounted for only on the basis of the nitrogen introduced with the water extracts.

Willaman [5] reported that peptone has the power to promote the growth of *Sclerotinia cinerea* in a pure salt and sugar medium. Since *Ophiobolus graminis* can utilize peptone, casein, and egg albumen as sources of nitrogen, they were tested in small amounts as sources of growth-promoting material. They were used in connection with ammonium chloride, sodium nitrate, glycine, uric acid, and gelatin; the control was without nitrogen. In no case was extra growth nóted except that which could be accounted for by the nitrogen added in the peptone, casein, or egg albumen.

Czapek's medium having urine as a source of nitrogen supported a good growth of *Ophiobolus graminis*. However, small additions of urine to Czapek's medium containing ammonium chloride did not make the ammonium chloride available. Accordingly, urine does not contain any growth-promoting material for *O. graminis* with respect to ammonium chloride.

NITROGEN PRESENT IN PLANT MATERIALS

Ophiobolus graminis will grow on a large number of different plant decoctions as well as on vegetable tissue. The following is a partial list of decoctions showing the diversified types on which it will grow: Onion, carrot, potato, wheat leaves, sweetpotato, dandelion leaves, radish tops and roots, bluegrass leaves, elm leaves, green beans, iris leaves, and spinach leaves. Approximately 300 g of each of these materials was boiled in 1,000 cc of water to extract the juices, and then dextrose was added at the rate of 20 g per liter. Onion, carrot, and potato tissue are favorable media. Wheat, barley, and oat seed also support good growth. It is probable that the nitrogen consumed in plant decoctions is of a proteinaceous nature.

SUMMARY

Ophiobolus graminis, the parasite causing take-all of wheat, has been found to be specific in its nitrogen food requirements when the nitrogen was supplied in a modified Czapek's nutrient solution. Egg albumen, casein, peptone, and nucleic acid were the only compounds utilized by the fungus. The nitrogen of the other materials tried, both inorganic and organic, was unavailable. This unavailability was not affected by the hydrogen-ion concentration of the media, by the source of carbon, nor by the presence of inorganic or organic growth-promoting materials. Many plant decoctions as well as the tissue of several plants were favorable media for *O. graminis*.

Rhizopus sp. and *Penicillium* sp. were specific in some cases as to the kind of nitrogen compound they could utilize. However, they could utilize many compounds that were unavailable to *Ophiobolus graminis*.

⁵ WILLAMAN, J. J.   THE FUNCTION OF VITAMINES IN THE METABOLISM OF SCLEROTINIA CINERKA.   Jour. Amer. Chem. Soc. 42: 549–585, illus. 1920.

THE INSECTICIDAL ACTION OF ACID LEAD ARSENATE ON THE LARVAE OF THE JAPANESE BEETLE IN DIFFERENT TYPES OF SOIL[1]

By WALTER E. FLEMING, *entomologist*, FRANCIS E. BAKER, *assistant entomologist*, and LOUIS KOBLITSKY, *junior chemist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

In a previous report from this laboratory (*10*) [2], it was shown that acid lead arsenate is the most dependable of the inorganic arsenicals for killing larvae of the Japanese beetle (*Popillia japonica* Newman) in the soil. The larvae are killed by ingesting a fatal dose of the arsenical as they burrow through the poisoned soil, or by feeding on rootlets growing in the soil. The time required for the material to be effective depends upon the stage of development, the degree of activity, and the susceptibility of the larvae. It is also influenced by the type of soil, for it has frequently been observed in the field that larvae of the Japanese beetle are more readily destroyed by acid lead arsenate in certain types of soils than in others. During the spring of 1935 a study was made of types of soil representative of the area within 100 miles of Moorestown, N. J., to determine whether the insecticidal effectiveness of the material was correlated with any intrinsic property of the soil.

DESCRIPTION OF THE SOILS

The soils used in this study included samples of soils developed from glacial till, soils developed from materials formed in situ by decomposition of underlying rocks, and soils transported from elsewhere and laid down by water. They were taken from various sections of New Jersey (*6, 7, 8, 11, 12, 13, 14, 15, 16, 17*), and can be broadly classified, according to physiographic location, as follows: Soils of the glaciated region, limestone valley soils, Piedmont Plateau soils, and Coastal Plain soils. These groups are further divided into soil series on the basis of structure, color, and drainage characteristics. The 15 types of soils selected for this study are described below.

SOILS OF THE GLACIATED REGION

Dover loam consists of a brown to light-brown, gritty loam, 8 to 15 inches deep, overlying yellowish-brown, gritty clay or gritty clay loam, which passes quickly into yellowish or reddish-yellow, friable sandy clay. It is derived from glacial till. Gravel and occasional boulders of limestone, gneiss, quartzite, and chert are present. Outcrops of limestone occur in many places. The soil is well drained.

Washington loam is a brown, mellow loam, 10 to 15 inches deep, overlying reddish-yellow, moderately friable silty clay, which usually is gritty, containing small fragments of partly decomposed rock.

Ophiobolus graminis was grown on potato-dextrose agar in Petri dishes. The agar with the adhering mycelium was cut into small blocks and one block was placed in each flask. The temperature of the growing cultures was maintained at 24° C. in an electric incubator. For the most part, growth observations were made by the unaided eye, and the relative abundance of growth was rated from none to the best by the symbols 0, 1, 2, 3, 4, and 5. In a few instances the mycelium was dried and weighed.

The nitrogen sources used consisted of both inorganic and organic compounds, as nearly chemically pure as can be purchased on the market. The organic nitrogen compounds included proteins of various complexity, representative members of the aliphatic and aromatic nitrogen series, the alkaloids, and miscellaneous compounds.

Certain nitrogen compounds probably are toxic to all forms of cryptogams. A pinch of garden soil was added to one flask of each type of culture to find whether any of the many organisms present would grow. Lack of all growth should be an indication of general toxicity. *Rhizopus* sp. and *Penicillium* sp., two vigorous growth types, were sown in other flasks for the same purpose.

It was necessary to exercise caution concerning the concentration of certain compounds that were being tested for the first time in a nutrient solution. A concentration that would permit growth was attained by adding to the culture solution containing the untried source of nitrogen a small amount of casein, which is utilized by *Ophiobolus graminis*. Growth under these conditions was taken to indicate that the material was not toxic, or if so, that the concentration was not great enough to be injurious. Two grams of any of the compounds tried in 1,000 cc of the medium was found not to be too concentrated.

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Ammonium sulphate	9	0	5	4	5 F.
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Calcium nitrate	2	0	0	5	5 B.
Magnesium nitrate	2	0	0	4	5 F.
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Sodium nitrate	5	0	5	5	5 F.
Sodium nitrite	2	0			5 F.
Organic:					
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Acetanilide	3	0			0.
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Albumen, egg	6	5			
Allantoin	2	0	5	2	5 B.
Asparagine	2	0	5	3	5 B.
Aspartic acid	2	0	5	3	5 B.
Benzenesulphonamid	2	0	0	0	T F.
Benzylamine	2	0	0	0	0.
Betaine hydrochloride	2	0	0	0	1 B F.
Caffeine	5	0	1		
Casein	Many	4			
Choline hydrochloride	2	0	2	3	5 B F.
Creatine hydrate	2	0	0	2	5 B.
Cyanamide	4	0			0.
Dicyandiamide	2	0	0	0	0.
Dinitrobenzoic acid	2	0	0	0	T F.
Diphenylamine	4	0			0.
Gelatin	2	0	3	2	3 B.
Glutamic acid	2	0	5	5	5 B.
Glycine	7	0	5	3	5 B.
Guanidine carbonate	2	0	0	4	5 B.
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Nicotine	4	0	0		5 F.
Nucleic acid	2	2			
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Phenylalanine	2	0	4	3	5 F.
Taurine	2	0	2	3	5 F B.
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Urea	2		50	4	5 F B.
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EFFECT OF SOURCES OF CARBON

Dextrose, sucrose, maltose, starch, dextrin, lactose, *d*-fructose, and *l*-xylose were added separately to portions of several batches of media, each batch containing a different source of nitrogen. The nitrogen compounds were egg albumen, casein, peptone, sodium nitrate, ammonium chloride, ammonium phosphate, potassium nitrate, and calcium nitrate. *Ophiobolus graminis* grew well in all the media containing egg albumen, casein, and peptone, but not in the media containing other nitrogenous sources irrespective of the source of carbon. Weight measurements of the growth of *O. graminis* were made with dextrose, sucrose, maltose, starch, dextrin, and lactose when used in

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Separate batches of media containing the nitrogenous compounds potassium nitrate, ammonium sulphate, ammonium dihydrogen phosphate, ammonium oxalate, and glycine were each subdivided into five parts. A trace of a compound of copper, sodium, silicon, zinc, and aluminum was added separately to each subdivision. In no case did the growth of *Ophiobolus graminis* in these cultures differ from that of the checks containing the several nitrogen compounds without the addition of these elements.

Brenner [3] mentioned that traces of ammonium salts have been found to aid in the assimilation of the alkaloids by *Aspergillus niger* and *Penicillium glaucum*. Traces of ammonium chloride, ammonium tartrate, ammonium formate, and ammonium phosphate were therefore added separately to different samples of media containing caffeine and nicotine. In no case was the growth of *Ophiobolus graminis* improved. Both caffeine and nicotine as a source of nitrogen in Czapek's solution will support certain soil fungi and bacteria, but not *O. graminis*.

Mockeridge [4] did considerable work with accessory factors or growth-promoting substances for green plants. He found that water-soluble material from leafmold, well-rotted manures, fertile manured soils, and well-decayed peat greatly promote the growth of *Lemna major* in nutrient solution. This was not due solely to nutritive action. The writer tried a similar type of experiment with *Ophiobolus graminis*, using a few cubic centimeters of water extracts from garden soil, leafmold, stable manure, chicken manure, and peat to 50 cc of the medium containing the nitrogen compound to be tested. The source of nitrogen in the medium was ammonium chloride. The media were steam sterilized and the organisms were then added. Any

[3] BRENNER, W. DIE STICKSTOFFNAHRUNG DER SCHIMMELPILZE. Centbl. Bakt. [etc.] (II) 40: 555–64 7 illus. 1914.
[4] MOCKERIDGE, F. A. THE OCCURRENCE AND NATURE OF THE PLANT GROWTH-PROMOTING SUBSTANCES IN VARIOUS ORGANIC MANURIAL COMPOSTS. Biochem. Jour. 14: [432]–450. 1920.

growth that occurred could be accounted for only on the basis of the nitrogen introduced with the water extracts.

Willaman [5] reported that peptone has the power to promote the growth of *Sclerotinia cinerea* in a pure salt and sugar medium. Since *Ophiobolus graminis* can utilize peptone, casein, and egg albumen as sources of nitrogen, they were tested in small amounts as sources of growth-promoting material. They were used in connection with ammonium chloride, sodium nitrate, glycine, uric acid, and gelatin; the control was without nitrogen. In no case was extra growth noted except that which could be accounted for by the nitrogen added in the peptone, casein, or egg albumen.

Czapek's medium having urine as a source of nitrogen supported a good growth of *Ophiobolus graminis*. However, small additions of urine to Czapek's medium containing ammonium chloride did not make the ammonium chloride available. Accordingly, urine does not contain any growth-promoting material for *O. graminis* with respect to ammonium chloride.

NITROGEN PRESENT IN PLANT MATERIALS

Ophiobolus graminis will grow on a large number of different plant decoctions as well as on vegetable tissue. The following is a partial list of decoctions showing the diversified types on which it will grow: Onion, carrot, potato, wheat leaves, sweetpotato, dandelion leaves, radish tops and roots, bluegrass leaves, elm leaves, green beans, iris leaves, and spinach leaves. Approximately 300 g of each of these materials was boiled in 1,000 cc of water to extract the juices, and then dextrose was added at the rate of 20 g per liter. Onion, carrot, and potato tissue are favorable media. Wheat, barley, and oat seed also support good growth. It is probable that the nitrogen consumed in plant decoctions is of a proteinaceous nature.

SUMMARY

Ophiobolus graminis, the parasite causing take-all of wheat, has been found to be specific in its nitrogen food requirements when the nitrogen was supplied in a modified Czapek's nutrient solution. Egg albumen, casein, peptone, and nucleic acid were the only compounds utilized by the fungus. The nitrogen of the other materials tried, both inorganic and organic, was unavailable. This unavailability was not affected by the hydrogen-ion concentration of the media, by the source of carbon, nor by the presence of inorganic or organic growth-promoting materials. Many plant decoctions as well as the tissue of several plants were favorable media for *O. graminis*.

Rhizopus sp. and *Penicillium* sp. were specific in some cases as to the kind of nitrogen compound they could utilize. However, they could utilize many compounds that were unavailable to *Ophiobolus graminis*.

[5] WILLAMAN, J. J.   THE FUNCTION OF VITAMINES IN THE METABOLISM OF SCLEROTINIA CINEREA.   Jour. Amer. Chem. Soc. 42: 549-585, illus.   1920.

THE INSECTICIDAL ACTION OF ACID LEAD ARSENATE ON THE LARVAE OF THE JAPANESE BEETLE IN DIFFERENT TYPES OF SOIL[1]

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INTRODUCTION

In a previous report from this laboratory *(10)* [2], it was shown that acid lead arsenate is the most dependable of the inorganic arsenicals for killing larvae of the Japanese beetle (*Popillia japonica* Newman) in the soil. The larvae are killed by ingesting a fatal dose of the arsenical as they burrow through the poisoned soil, or by feeding on rootlets growing in the soil. The time required for the material to be effective depends upon the stage of development, the degree of activity, and the susceptibility of the larvae. It is also influenced by the type of soil, for it has frequently been observed in the field that larvae of the Japanese beetle are more readily destroyed by acid lead arsenate in certain types of soils than in others. During the spring of 1935 a study was made of types of soil representative of the area within 100 miles of Moorestown, N. J., to determine whether the insecticidal effectiveness of the material was correlated with any intrinsic property of the soil.

DESCRIPTION OF THE SOILS

The soils used in this study included samples of soils developed from glacial till, soils developed from materials formed in situ by decomposition of underlying rocks, and soils transported from elsewhere and laid down by water. They were taken from various sections of New Jersey (*6, 7, 8, 11, 12, 13, 14, 15, 16, 17*), and can be broadly classified, according to physiographic location, as follows: Soils of the glaciated region, limestone valley soils, Piedmont Plateau soils, and Coastal Plain soils. These groups are further divided into soil series on the basis of structure, color, and drainage characteristics. The 15 types of soils selected for this study are described below.

SOILS OF THE GLACIATED REGION

Dover loam consists of a brown to light-brown, gritty loam, 8 to 15 inches deep, overlying yellowish-brown, gritty clay or gritty clay loam, which passes quickly into yellowish or reddish-yellow, friable sandy clay. It is derived from glacial till. Gravel and occasional boulders of limestone, gneiss, quartzite, and chert are present. Outcrops of limestone occur in many places. The soil is well drained.

Washington loam is a brown, mellow loam, 10 to 15 inches deep, overlying reddish-yellow, moderately friable silty clay, which usually is gritty, containing small fragments of partly decomposed rock.

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Angular fragments and pebbles of gneiss, quartzite, flint, and occasionally limestone are present. The parent material consists of old glacial drift, in most cases overlying limestone or shale.

Dutchess shale loam is a light-brown to grayish-brown silt loam, 6 to 10 inches deep. The subsoil ranges from a yellowish-brown silt loam to silty clay loam. The depth to bedrock probably does not average more than 2 feet, and shale outcrops in places on tops of hillocks and crests of ridges. The rock fragments in the soil vary from small chips to platy pieces 4 to 5 inches across. Foreign boulders and gravel of quartzite and gneiss and occasionally limestone occur. The soil is well drained; in fact, crops often suffer from lack of moisture during dry periods, especially where the bedrock is close to the surface.

LIMESTONE VALLEY SOILS

Hagerstown silt loam was the only soil obtained from the limestone valley. The soils of this locality have been derived from limestone, and represent the residual material left upon removal in solution of the calcium and magnesium carbonates. The soil is thus composed of impurities in the original rock. The surface soil is a brown, mellow silt loam, 10 to 15 inches deep, overlying slightly reddish yellow, moderately friable silty clay, which shows no important change to a depth of 3 feet or more. Fragments and small outcroppings of limestone are of common occurrence. The topography is gently rolling and the drainage excellent.

PIEDMONT PLATEAU SOILS

Penn shale loam consists of Indian-red silt loam, 8 to 14 inches deep, over brighter Indian-red, brittle clay derived from Triassic formations. There is an abundance of shale fragments from the surface down, and usually the parent shale occurs within the upper 3-foot layer. Often the lower subsoil is friable, owing to the larger quantity of partly decomposed shale material present. Shale fragments range from less than one-eighth to one-half inch or more in thickness and have practically the same color as the soil. This soil occupies gently rolling ridges, hillsides, and stream slopes. Run-off is excessive, and crops often suffer from lack of moisture, especially during dry seasons.

Chester gravelly loam is a light-brown or brown, gritty loam, 8 to 12 inches deep, overlying brownish-yellow or yellow, friable, gritty clay loam or clay. Angular fragments of gneiss are abundant over the surface and through the soil and subsoil, giving the former a decidedly gravelly character. Varying quantities of mica are found. In places the soil is shallow, and the yellowish subsoil comes near enough to the surface to give plowed fields a spotted brownish and yellowish appearance. This soil is derived from the weathered products of coarse-grained gneiss of pre-Cambrian age. Fragments of parent rock ranging in size from huge masses to small gravel are encountered. The soil is well drained, except in a very few places bordering depressions on ridges, where the underdrainage is imperfect.

COASTAL PLAIN SOILS

The soil-forming materials of the Coastal Plain in New Jersey consist of unconsolidated water-laid sands, clay, gravel, greensand, and marly clay. The beds are composed of materials that were trans-

ported from older land areas and deposited, in part, under marine conditions. Since the elevation of this part of the ocean floor, streams, winds, and other agencies of erosion have removed some of the materials and more or less modified and rearranged the original deposits.

Sassafras sand under virgin conditions consists of a layer of gray to brown sand, about 1 inch thick, underlain by a brownish-gray sand, which at a depth of 5 to 8 inches passes into orange or reddish-yellow slightly loamy sand subsoil. The drainage is good to excessive, and in dry seasons crops are likely to suffer greatly from drought.

Sassafras sandy loam consists of a light-brown or brown sandy loam or loamy sand from 6 to 12 inches deep. This rests on orange-yellow or reddish-yellow sandy loam, which at a depth of 20 inches or less passes into yellowish-red, reddish-yellow, or dull-red friable sandy clay, generally becoming lighter in texture at a depth of about 3 feet. The soil is considered one of the most productive for general farm crops. It is easy to work and holds up well during protracted dry spells.

Collington sandy loam consists of brown loamy sand or sandy loam from 8 to 12 inches deep, underlain by yellowish-brown friable sandy clay continuous in most places to a depth of more than 3 feet. The subsoil contains enough greensand marl to give it a decidedly greenish cast. The soil is easy to work and appears to withstand protracted periods of dry weather.

Colts Neck loam is red or brownish-red loam to a depth of 7 or 8 inches. The subsoil is red or reddish-brown friable sandy clay loam or sandy clay. In the lower part of the subsoil, usually below a depth of 30 inches, the texture becomes somewhat lighter, the structure more porous, and the color changes gradually to a yellowish red. The Colts Neck soils are characterized by the presence of ferruginous fragments consisting usually of what are known as "clay ironstones." There is wide variation in the number as well as in the size and shape of these fragments. In the level areas they are few and very small, resembling the fragments of shale in soils derived from shale material. In some spots, however, they may compose as much as 30 to 40 percent of the soil mass, and attain a weight of several hundred pounds. The soil is well drained.

Keyport loam is a grayish-brown loam to a depth of 12 inches. It is underlain by yellow moderately friable clay loam, which, at a depth ranging from 15 to 24 inches, grades into somewhat plastic clay containing gray and yellow spots indicative of incomplete oxidation. Quartz particles may be present on the surface. Both the subsoil and the substratum are comparatively heavy and in wet weather are subject to excessive ground water, but this is soon disposed of because of the comparatively good surface drainage.

Lakewood sand is light-gray or white loose sand ranging from 6 to 24 inches in thickness. In the untilled soil there is in most places a thin layer beneath the forest mold which contains enough organic matter to give the sand a darker color. The subsoil is yellow, coffee-brown, or golden-yellow sand, and in most cases is uniform in character to a great depth. Lakewood sand is easily cultivated, but the crops suffer from drought.

Lakewood sandy loam, deep phase, consists of about 4 to 8 inches of white sand, frequently a little grayish in the surface inch owing to the presence of organic matter, underlain by yellow or orange-yellow loamy sand, passing, at depths ranging from about 12 to 24 inches into reddish-yellow, orange-yellow, or yellow sandy loam to sandy clay, which, at depths of about 30 inches, passes abruptly into yellow friable sandy clay. This type occupies high, well-drained areas with rolling topography. The drainage is thorough to excessive. Practically none of the soil is cleared. The scrubby forest covering it consists of scrub oak and pine with an undergrowth of bracken and huckleberry.

Elkton silt loam is a grayish silt loam from 5 to 12 inches deep, underlain by bluish-gray or almost white silty loam which, at a depth varying from 15 to 20 inches, grades to light bluish-gray silty clay loam mottled with yellow or rust brown. The surface soil is slightly compact and contains very little organic matter, even in timbered areas. The soil occupies flat or depressed areas and is generally poorly drained. Early in the spring much of it is so covered with water that the areas resemble ponds. These become dry as the season advances.

Woodstown sandy loam consists of a light-brown or brown loamy sand, sandy loam, or heavy sandy loam underlain at a depth of about 6 to 8 inches by a yellowish sandy loam or heavy sandy loam which in places shows a slight reddish cast. At a depth between 20 and 24 inches mottled yellow and light-gray or bluish-gray sandy clay is reached. This becomes more sandy below, and coarse sand and gravel occur at a depth of less than 40 inches. The soil occupies flat, low areas, mainly about stream heads and near areas of tidal wash. Underdrainage is imperfect and water is found in many places within the 3-foot depth.

DETERMINATION OF THE COEFFICIENTS OF EFFECTIVENESS OF ACID LEAD ARSENATE IN DIFFERENT TYPES OF SOIL

Each soil used in this study was obtained from the locality in New Jersey in which it predominated. As far as possible, the samples were taken from pastures, wood lots, and fields that were not under cultivation, as such soils had not been much modified from their natural condition.

In the field differences in texture, moisture content, and vegetation make it difficult to obtain a uniform distribution of the poison. This, together with the wide variation in the density of the larval population, introduces complications which practically preclude any satisfactory comparison of the soils under natural conditions without extensive experimentation. Many of these factors can be controlled in the laboratory. By placing the soils in earthen pots and maintaining them at their best moisture content at a constant temperature, by having the same vegetation growing in each soil, and by introducing the same number of larvae in the same stage of development and from the same source, and leaving them in the soil for the same length of time, as outlined by Fleming and Baker (*10*), it is possible to compare the insecticidal action of the material in the different soils without extensive work in the field.

ach soil was treated with different concentrations of acid lead arsenate, ranging from 250 to 2,000 pounds per acre, to determine the concentrations that would kill 50 percent of the larvae. Two hundred third-instar larvae were used for each dosage. The effectiveness of acid lead arsenate in the different soils was expressed as the coefficient of effectiveness in Sassafras sandy loam, which was used as a standard soil.

The coefficients of effectiveness, arranged in order of decreasing effectiveness, are given in the following tabulation:

	Coefficient of insecticidal effectiveness
Soil type:	
Lakewood sand	2. 18±0. 56
Sassafras sand	2. 02± . 36
Woodstown sandy loam	1. 14± . 19
Lakewood sandy loam	1. 12± . 16
Dutchess shale loam	1. 01± . 13
Sassafras sandy loam	1. 00± . 13
Washington loam	. 98± . 15
Dover loam	. 97± . 15
Hagerstown silt loam	. 86± . 10
Keyport loam	. 81± . 12
Chester gravelly loam	. 69± . 10
Elkton silt loam	. 69± . 10
Penn shale loam	. 65± . 07
Collington sandy loam	. 46± . 07
Colts Neck loam	. 30± . 05

It is apparent that the effectiveness of lead arsenate as a stomach poison varies in the different soils. In a previous report (*10*) it was shown that the arsenates of aluminum, barium, calcium, iron, lead, magnesium, manganese, and zinc were toxic to Japanese beetle larvae. Recently it was found that such lead compounds as the acetate, borate, carbonate, chloride, fluoride, and acid phosphate were not toxic to the larvae, even when applied at the rate of 2,000 pounds per acre. It may be concluded, therefore, that the insecticidal action of lead arsenate on this insect in the soil is due entirely to the arsenate radical and that the variation in the toxicity of the compound in different soils is due to the reaction of the arsenate radical with the colloidal matter or salts in the soil to form other arsenates of varying toxicity.

CORRELATION BETWEEN INSECTICIDAL ACTION OF ACID LEAD ARSENATE AND CERTAIN CONSTITUENTS OF THE SOIL

The total content of certain soil constituents in the various soils (*1, 2, 3, 4, 5*) is given in table 1. A multiple-correlation analysis was made of the data, according to the procedure outlined by Ezekiel (*9*), to determine the extent to which the variation in the insecticidal action was associated with the variation in the total nitrogen, phosphoric acid, potassium, calcium, magnesium, and carbon in the soils. It was found that the correlation between the coefficient of insecticidal effectiveness and the total plant food was insignificant.

TABLE 1.—*Average quantities of certain soil constituents in the surface soil of the types used in the present study*

Soil type	Nitrogen (N)	Phosphoric acid (P₂O₅)	Potassium (K₂O)	Calcium (CaO)	Magnesium (MgO)	Carbon (C)
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Lakewood sand	300	50	340	2, 080	970	2, 232
Sassafras sand	560	740	3, 890	1, 410	1, 710	3, 520
Lakewood sandy loam	150	100	430	350	140	3, 940
Dutchess shale loam	1, 870	2, 610	17, 700	3, 260	16, 080	20, 890
Sassafras sandy loam	1, 020	1, 180	8, 050	2, 610	4, 130	8, 980
Washington loam	1, 135	1, 195	23, 405	3, 975	7, 440	12, 500
Dover loam	1, 790	1, 740	38, 160	6, 790	11, 040	20, 079
Hagerstown silt loam	1, 360	1, 800	58, 390	7, 440	15, 680	14, 050
Keyport loam	1, 330	970	11, 490	1, 190	3, 040	14, 140
Chester gravelly loam	2, 135	2, 090	23, 360	10, 560	10, 230	22, 200
Elkton silt loam	1, 190	650	12, 190	4, 130	2, 030	10, 300
Penn shale loam	1, 800	1, 310	21, 280	4, 060	17, 520	17, 010
Collington sandy loam	990	3, 130	22, 940	5, 040	10, 130	9, 390
Colts Neck loam	1, 180	9, 370	16, 010	1, 490	3, 070	14, 070

CORRELATION BETWEEN INSECTICIDAL ACTION OF ACID LEAD ARSENATE AND CONTENT OF WATER-SOLUBLE CONSTITUENTS AND ACIDITY OF THE SOIL

The different soils were analyzed according to the procedure outlined by Spurway (*18*) to determine the quantities of water-soluble soil constituents. The hydrogen-ion concentration was determined by the potentiometer. Negative tests were obtained for water-soluble carbonates, nitrites, sulphates, aluminum, ferrous iron, and sodium. The positive results are given in table 2. .

TABLE 2.—*Coefficient of effectiveness, hydrogen-ion concentration, and content of water-soluble constituents of the different types of soil used in the multiple-correlation analysis*

Soil type	Coefficient of effective- ness (X_1)	pH (X_2)	Chlo- rides (X_3)	Ni- trates (X_4)	Phos- phates (X_5)	Ammo- nia (X_6)	Cal- cium (X_7)	Mag- nesium (X_8)	Potas- sium (X_9)	Man- ganese (X_{10})
			P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m*
Lakewood sand	2. 18±0. 56	4. 59	0	10	2. 50	2	30	5	5	0
Sassafras sand	2. 02± . 36	4. 30	10	20	1. 00	10	60	1	5	1
Woodstown sandy loam	1. 14± . 19	5. 73	10	20	. 50	1	100	7	5	0
Lakewood sandy loam	1. 12± . 16	4. 61	0	5	1. 00	3	0	3	5	0
Dutchess shale loam	1. 01± . 13	5. 53	5	10	. 00	10	90	7	5	5
Sassafras sandy loam	1. 00± . 13	4. 96	5	20	. 75	1	20	3	0	1
Washington loam	. 98± . 15	7. 46	20	15	. 00	2	150	7	10	5
Dover loam	. 97± . 15	6. 08	5	5	. 50	2	40	7	20	2
Hagerstown silt loam	. 86± . 10	7. 38	0	20	. 50	2	100	7	20	1
Keyport loam	. 81± . 12	5. 05	10	5	. 00	7	100	7	20	0
Chester gravelly loam	. 69± . 10	5. 91	20	20	. 75	1	100	7	10	1
Elkton silt loam	. 69± . 10	4. 27	15	20	. 50	1	0	0	0	0
Penn shale loam	. 65± . 07	6. 03	0	20	. 75	2	40	7	5	2
Collington sandy loam	. 46± . 07	4. 61	5	15	. 00	5	40	7	20	1
Colts Neck loam	. 30± . 05	5. 45	30	10	. 00	3	60	7	20	1

A multiple-correlation analysis was made of the data to determine the extent to which the variation in the effectiveness of acid lead arsenate as an insecticide was associated with the variation in the

hydrogen-ion concentration and with the various water-soluble con-stituents of the soil. The coefficient of multiple correlation, $\bar{R}$, adjusted for the number of cases, was 0.877; the coefficient of multiple determination, $\bar{R}^2$, was 0.769, and the adjusted standard error of estimate, $\bar{S}$, was 0.244. Since the coefficient of multiple determination showed that about 77 percent of the variance in the insecticidal action was associated with the water-soluble constituents of the soils, it is apparent that the water-soluble constituents of the soil have the greatest influence on the effectiveness of an insecticide.

The net regression coefficients, the beta net regression coefficients, the coefficients of part correlation, and the coefficients of part deter-mination were calculated according to the procedure outlined by Ezekiel (9), and are given in table 3. It was found that an increase in water-soluble phosphates, calcium, manganese, ammonia, or potassium increased the effectiveness of acid lead arsenate as an insecticide, and that an increase in magnesium, hydrogen-ion concentration, chlorides, or nitrates decreased its effectiveness.

TABLE 3.—*Net regression coefficients, coefficients of part correlation, coefficients of part determination, and beta net regression coefficients obtained from correlating the coefficients of insecticidal effectiveness with the proportion of water-soluble constituents and the acidity in various types of soil*

Independent variable	Net regression coefficient [1]	Coefficient of part correlation	Coefficient of part determination	Beta net regression coefficient	Percent or weight of the beta coefficients
Phosphates (X_5)	0.676004	0.873	76.1	0.858	28.0
Calcium (X_7)	.008328	.828	68.5	.709	23.1
Magnesium (X_8)	−.104969	.729	53.1	−.511	16.7
Chlorides (X_3)	−.012966	.427	18.3	−.227	7.4
pH (X_2)	−.114845	.426	18.3	−.226	7.4
Manganese (X_{10})	.056973	.356	12.7	.183	6.0
Nitrates (X_4)	−.010381	.256	6.6	−.127	4.2
Ammonia (X_6)	.020212	.249	6.2	.124	4.0
Potassium (X_9)	.006452	.202	4.1	.099	3.2

[1] In terms of the coefficient of insecticidal effectiveness. The value of the constant term for the net regression equation is 1.337608.

The importance of each of these independent variables may be measured by the coefficient of part correlation and by the coefficient of part determination. In table 3 it is seen that the variation in con-centration of phosphates in the soil accounted for 76 percent of the variation in insecticidal effectiveness of acid lead arsenate not ex-plained by variation in the concentrations of the other water-soluble constituents; similarly, that calcium accounted for 68 percent, and that magnesium accounted for 53 percent. The variation in the hydrogen-ion concentration or in the concentration of chlorides, manganese, nitrates, ammonia, or potassium was a minor factor in modifying the insecticidal action.

The net regression coefficients show the average change in effective-ness of acid lead arsenate which occurs with each increase of specified units of hydrogen-ion concentration, or in the water-soluble constit-uents of the soil. The size of the regression coefficients, however, varies not only with the relation between the variables but also with the units by which each variable is expressed. These coefficients can be reduced to another form by stating each of the variables in

units of its own standard deviation. In this form the net regression coefficients are termed "beta" coefficients and the relative importance of each variable can be determined. When the beta coefficients are compared with the coefficients of part correlation, it is seen that in the two sets of measurements the order of importance of the nine variables is the same. The last column of table 3 indicates the percentage or weight of each independent factor in the whole correlation study. It is hardly to be expected, in a problem consisting of nine independent variables, that significant results can be obtained for each variable.

• SUMMARY AND CONCLUSIONS

Larvae of the Japanese beetle are killed by ingesting acid lead arsenate while burrowing through poisoned soil or feeding on rootlets growing in the soil. The insecticidal action may be attributed entirely to the arsenate radical, for arsenates of other metals are toxic, and other lead salts that were tested were nontoxic.

In a study of the effectiveness of acid lead arsenate in 15 types of soil from New Jersey, it was found that the coefficient of insecticidal action ranged from 0.30 in Colts Neck loam to 2.18 in Lakewood sand. The difference in the action of acid lead arsenate appeared to be associated with some intrinsic property of the soils. It is probable that the variation in toxicity is due to the reaction of the arsenate radical with the colloidal matter or salts in the soil to form arsenates of varying degree of toxicity.

There was an insignificant correlation between the coefficient of insecticidal effectiveness and the total nitrogen, phosphates, potassium, calcium, magnesium, and carbon in the soils.

The water-soluble constituents of the soils accounted for 77 percent of the variance in the insecticidal action. The concentrations of water-soluble phosphates, calcium, and magnesium appear to be the most important factors influencing the effectiveness of acid lead arsenate. The greater the concentrations of soluble phosphates and calcium in the soil when acid lead arsenate is applied, the more effective it is, and the greater the concentration of soluble magnesium the less effective it is. The influence of soluble chlorides, manganese, nitrates, ammonia, and potassium in the soil on insecticidal action is of minor importance. Although the hydrogen-ion concentration is a minor factor, the nature of the radicals producing the acidity or alkalinity being more important, it is apparent that acid lead arsenate is generally more effective in acid than in alkaline soils.

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INFLUENCE OF BLACK POINT DISEASE, SEED TREATMENT, AND ORIGIN OF SEED ON STAND AND YIELD OF HARD RED SPRING WHEAT [1]

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INTRODUCTION

In 1934 wheat seed carrying the black point disease was planted to determine what effect if any would be produced on the stand and yield of the progeny. In addition, a study was made of the effect of Ceresan as a seed treatment and the effect on subsequent yield and stand of inoculating seed with bunt spores. Yield and stand of plants from large seed grown in the greenhouse were compared with yield and stand of plants from smaller seed grown out of doors, and, finally, a comparison was made of some hybrid selections with one of the parents, Ceres. While the experiment may be somewhat complex, it is not believed to be less accurate because of the several factors considered. Indeed, Fisher and Wishart [2] advocate complex experiments wherever possible because of the very high degree of precision that may be attained in such experiments. In the present experiment the degrees of freedom for the error are 33. Thus the standard error was small in comparison with the mean, as will later appear.

BLACK POINT INFECTION AND SIZE OF KERNEL

The black point carried by the seed resulted from infection during the growing season of 1933. The infections were caused almost entirely by *Helminthosporium sativum* and an *Alternaria*.[3] Because of the serious nature of *Helminthosporium* infection in wheat it would appear undesirable to plant seed obviously infected with the organism. Güssow [4] reported that the seedlings from black point wheat appeared to be less vigorous than those from sound kernels. Henry [5], experimenting with Marquis wheat containing kernels infected with black point, found that germination was injured both in the greenhouse and outside. Two pairs of rod rows were planted by him in the nursery on different dates. The first pair, planted on May 20, showed a yield from the normal seed of 250 percent of that from the diseased seed. With the other pair, planted 5 days later, the difference was trifling. This experiment carried no replications.

Many experiments have been conducted to determine the influence of size of kernel on yields. Percival [6], experimenting with three

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varieties of wheat, planted kernels weighing 25 and 50 mg in each of 5 years and found that the larger kernels produced the larger yields. The gains in yield ranged from nearly 40 to 50 percent. In the present experiment the large kernels were grown under greenhouse conditions, and the smaller kernels out of doors. A review of the literature fails to show any comparison of growth and yield in a crop resulting from the planting of wheat kernels of different sizes which had been grown under outside and inside environments.

MATERIAL AND METHODS

The seed consisted of two sorts. One was the Ceres variety of wheat (*Triticum aestivum* L.) and the other was a composite sample of three hybrid selections of the F_6 seed of the cross Ceres × (Hope× Florence). The seed coming from the greenhouse was F_7 seed grown unselected from the F_6 seed. The additional generation would scarcely modify the genotype of the seed. As laboratory tests indicated high germinating capacity for both lots, any difference in results that might be obtained from the two lots could scarcely be due to inferior seed in one of the lots. Certain characters of the two lots are shown in table 1.

TABLE 1.—*Data on the seed lots used*

Variety or selection	Condition	Parent seed grown	Weight per 1,000 kernels	Relative weight of kernels	Seed used per acre
			Grams		*Pounds*
CeresX (HopeXFlorence)	Healthy	Outside	35. 5	112	85
Do	Diseased	do	37. 5	118	90
Do	Healthy	In greenhouse	49. 0	154	117
Ceres	do	Outside	31. 8	100	76

Seed of each of the four classes was given three kinds of treatment. One portion was planted as a control, another was treated before planting with New Improved Ceresan, and a third was inoculated with bunt spores. The seed for the first two classes, healthy and diseased, consisted of a single lot, and out of this a sufficient number of healthy and black point-infected kernels were hand picked for the experiment. The kernels classed as healthy bore no outward sign of infection.

The plantings were made in quadruplicate in 8-foot rows, 200 kernels being used for each row, and the rows were randomized. Random planting evidently should compensate for lack of guard rows in this experiment since different rates of seeding were immediately adjacent. Planting was done by hand on April 21, and emergence was in progress by April 30. A stand count was taken on May 5 and a second count on May 15. The first revealed no critical information as to relative quickness of germination.

STAND

The stand counts secured on May 15 in seedlings per 8-foot row are shown in table 2.

TABLE 2.—*Stand counts secured May 15, per 8-foot row, from 200 kernels planted*

Planting no.	Black point-infected			Healthy			Greenhouse-grown			Ceres variety			Average
	Control	Treated	Bunted	Control	Treated	Bunted	Control	Treated	Bunted	Control	Treated	Bunted	
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
1	159	164	180	168	171	167	198	193	182	166	172	164	174
2	175	165	171	170	173	180	190	198	194	180	173	179	179
3	162	163	168	166	173	176	188	193	192	179	178	169	176
4	171	169	161	168	180	170	191	195	193	170	163	177	176
Percentage of stand	83.4	82.6	85.0	84.0	87.1	86.6	95.9	97.4	95.1	86.9	85.8	86.1	

The data in table 2 were analyzed according to Fisher's [7] analysis of variance method. The only *z* value of significance was that for the four classes, and this at 1.98 was of high value, as would be expected from the relatively large stands shown from the greenhouse seed. The standard error was calculated as 5.3, which is 3.01 percent of the mean of 176. The significant difference for a mean of four rows is 8. The number of seedlings from bunt-inoculated seed in the first two classes was greater than for the control but not significantly so. For Ceres the seedlings from bunt-inoculated seed were essentially equal to the number of seedlings from seed treated with Ceresan. The average stands for the seven units are given in table 3. The number of seedlings from bunt-inoculated seed in the first two classes was greater than for the control but not significantly so. For Ceres the seedlings from bunt-inoculated seed were essentially equal to the number of seedlings from seed treated with Ceresan. The average stands for the seven units are given in table 3.

TABLE 3.—*Average stand counts secured May 5 and May 15 per 8-foot row from 200 kernels planted, using seed of kinds indicated*

Description of seed	Stand count on –		Description of seed	Stand count on –	
	May 5	May 15		May 5	May 15
	Number	Number		Number	Number
Black point-infected	139	167	Control	144	175
Healthy	148	172	Treated with Ceresan	145	176
Greenhouse-grown	161	192	Inoculated with bunt spores	153	176
Ceres variety	141	173			

Considering the stand count for the first four classes taken May 15, the value for significant difference is 4.6. Thus the black point seed had a significantly lower stand than the remaining lots. The stand from the greenhouse-grown seed is decidedly larger than for any of the others. Was this increased stand due entirely, or at all, to the larger seed? Other experiments, not reported here, indicate that the

[7] FISHER, R. A., and WISHART, J.   See footnote 2.

larger kernel of the seed from the greenhouse was not responsible for the better stand. The weight per 1,000 kernels of the greenhouse-grown seed was 154 percent of that of Ceres, while the weight of the healthy seed was 112 percent. The corresponding averages for the stand count of May 5, also given in table 3, agree fairly well with the count taken on May 15 when all, or nearly all, seedlings had emerged. The stand from the greenhouse-grown seed stands out prominently in the early count, as it does in the later one.

The average date of heading was June 17. Ceres headed nearly 1 day later than did the other wheats, a significant difference.

YIELD OF GRAIN

At harvest the plants were pulled and wrapped separately. Before threshing, counts were made of the number of plants, the number of heads, and the number of bunted heads. The single yields of threshed grain and the average yields in bushels per acre are given in table 4; the analysis of variance is shown in table 5.

TABLE 4.—*Yield of grain per acre secured from various types of seed*

Planting no.	Yield for seed type indicated												Aver- age
	Black point-infected			Healthy			Greenhouse-grown			Ceres variety			
	Con- trol	Treat- ed	Bunt- ed	Con- trol	Treat- ed	Bunt- ed	Con- trol	Treat- ed	Bunt- ed	Con- trol	Treat- ed	Bunt- ed	
	Bu.	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*
1	41.6	37.2	35.8	32.4	40.2	33.6	36.4	40.0	36.6	42.2	35.4	17.8	35.8
2	38.2	34.8	31.8	37.8	40.4	36.4	44.8	46.4	36.8	36.6	39.8	21.2	37.0
3	39.8	31.6	26.2	34.6	34.6	28.0	41.2	41.8	34.6	28.4	35.6	14.0	32.6
4	34.2	33.0	33.2	32.4	37.0	29.4	37.2	36.8	34.2	36.0	36.4	18.2	33.2
Average	38.5	34.2	31.8	34.3	38.1	31.9	39.9	41.3	35.6	35.8	36.8	17.8	34.6

TABLE 5.—*Analysis of variance for yield*

Due to—	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	z value
Replication	[1] 3	[1] 4,154	1,385		
Experimental conditions	[1] 11	[1] 40,442	3,677	1.8023	
Classes	3	11,546	3,849	1.8242	1.5139
Treatment	2	17,537	8,768	2.2368	1.9265
Interaction	6	11,359	1,893	1.4704	1.1601
Error	[1] 33	[1] 6,138	186	.3103	
Total	47	50,734			

[1] Items comprising total.

The standard error equals 2.73 bushels, which is 7.89 percent of the mean yield of 34.6 bushels. The differences in treatment and in classes are markedly significant as the z values indicate. The significant difference of a mean yield of 4 equals 4.1 bushels. The 12 yields in order are shown in table 6, as are also the averages for classes and treatments.

TABLE 6.—*Yields per acre as related to kind of seed used and treatment*

YIELDS TABULATED IN DESCENDING ORDER

Seed	Treatment	Yield	Seed	Treatment	Yield
		Bushels			*Bushels*
Greenhouse-grown	Ceresan	41.3	Healthy	Control	34.3
Do	Control	39.9	Black point-infected	Ceresan	34.2
Black point-infected	____do	38.5	Healthy	Bunt-inoculated	31.9
Healthy	Ceresan	38.1	Black point-infected	____do	31.8
Ceres variety	____do	36.8	Ceres variety	____do	17.8
Do	Control	35.8			
Greenhouse-grown	Bunt-inoculated	35.6	Average		34.6

AVERAGE YIELDS

Greenhouse-grown		38.9	Ceres variety		30.1
Black point-infected		34.8		Ceresan	37.6
Healthy		34.7		Control	37.1
				Bunt-inoculated	29.2

Bunt treatment enters into the yields of each of four classes listed in column 1 of table 6, but only Ceres is markedly affected. The significant difference for classes is 2.4 bushels. Thus it is evident that we have three groups significantly different from each other.

If the bunted rows are excluded from the calculations the efficiency of the results is lessened but comparisons are still worth while. The analysis of variance calculated with the 16 bunted rows omitted is given in table 7.

TABLE 7.—*Analysis of variance of yields with bunt-infected rows omitted*

Due to	Degrees of freedom	Sum of squares	Mean square	½ log.	z value
Replications	[1] 3	[1] 2,558	853		
Experimental conditions	[1] 7	[1] 4,567	652	0.9373	0.5287
Treatment	1	41	41		
Classes	3	2,797	932	1.1163	.7077
Interaction	3	1,729	576	.8757	.3672
Error	[1] 21	[1] 4,754	226	.4086	
Total	31	11,879			

[1] Items comprising total.

The average yield of the rows in which there was no bunt is 37.3 bushels per acre, and the standard error of a single yield is 3.01 bushels or 8.07 percent of the mean yield. The only z value which is significant and which is of interest in these comparisons is that for classes. Average yields now for the various class units are as follows:

	Bushels
Greenhouse-grown	40.6
Ceres variety	36.3
Black point-infected	36.3
Healthy	36.2
Average	37.3

The significant difference for this group is 3.2 bushels, that for the group from greenhouse-grown seed. The remaining yields are nearly identical. So far as could be judged by appearance, the greenhouse seed differed from the healthy and Ceres seed only in size and greater

plumpness. It would seem that if seed size had been the only factor involved in the yield increase there would have been some disparity in yield between Ceres and the other two groups due to difference in seed weight. But the yields from Ceres and the hybrids are essentially the same.

Other data should indicate what characters of the plant contributed to the high yield of the wheat from greenhouse seed. Such data are given in table 8, which shows the percentage of the seedling stand

TABLE 8.—*Stand maturing, mature plants, stooling, and grain yield per plant for various types of seed planted* [1]

Seed	Stand maturing	Mature plants per 200 seeds	Stools per plant	Grain per plant
	Percent	*Number*	*Number*	*Grams*
Greenhouse-grown	92.8	179	1.8	1.13
Healthy	93.5	160	1.8	1.13
Black point-infected	93.1	155	1.9	1.17
Ceres variety	94.1	163	1.7	1.12

[1] Bunted plants omitted.

that matured, the number of mature plants, the degree of stooling, and the weight of grain per plant. The bunted plants are omitted for a more valid comparison.

As compared with the plants from the greenhouse-grown seed the plants from the healthy seed showed no compensation for reduced stand by increased stooling or more grain ·per plant. Consequently the greenhouse-grown seed showed the same percentage excess in yield of grain that it had in respect to stand. If there had been a compensation in both instances one would expect the stooling from the healthy seed to be larger than that from the greenhouse seed because of fewer plants, and the grain per plant to be greater from the greenhouse seed because of the greater vigor of the plants. Evidently such was not the case.

It is evident from the foregoing data that it was the larger initial stand that provided the greater yield of that portion of the crop grown from the greenhouse seed.

While Ceres showed decidedly greater susceptibility to bunt than did the other wheats, the yields of the Ceres × (Hope × Florence) selections evidently were also affected. The percentage of mature plants secured from the stand of May 15 from the clean and from the bunt-inoculated seed, the grain per plant, and the yields are shown in table 9.

TABLE 9.—*Percentages of mature plants from stand of May 15, weight of grain per plant, and associated yields, for clean and bunt-inoculated seed of various types*

Seed	Mature plants from stand		Grain per plant		Yields per acre		
	Clean	Bunted	Clean	Bunted	Clean	Bunted	Decrease
	Percent	*Percent*	*Grams*	*Grams*	*Bushels*	*Bushels*	*Bushels*
Greenhouse-grown	92.8	89.5	1.13	1.05	40.6	35.6	5.0
Healthy	93.5	92.0	1.13	1.00	36.2	31.9	4.3
Black point-infected	93.1	92.5	1.17	1.01	36.4	31.8	4.6
Ceres variety	94.1	78.0	1.12	.66	36.3	17.8	18.5

*The loss of plants from the seedling stage to maturity among the bunted rows is not significant except for Ceres. The differences in amount of grain per plant are significant except from the greenhouse seed. The differences in yield are significant in all cases. It is evident from table 9 that the loss among the plants from black point-infected seed from May 15 on was no greater than that among the plants from healthy seed. The loss of stand among the plants from seed treated with bunt was inappreciable except for the Ceres variety. In that case the loss was severe.

COMPARISON OF SEED TREATMENTS

The effect of treatment with Ceresan and inoculation with bunt spores on stand and other characters is shown in table 10.

TABLE 10.—*Effect of Ceresan treatment and inoculation with bunt spores on stand, number of mature plants produced, stooling, and yield*

Seed treatment	Stand maturing	Mature plants per 200 seeds	Stools per plant	Grain per plant
	Percent	*Number*	*Number*	*Grams*
Control	93.9	163	1.8	1.14
Ceresan	92.8	166	1.8	1.13
Bunt-inoculated	88.0	155	1.8	.93

Treatment with Ceresan evidently had no appreciable effect on the characters shown; inoculating the seed with bunt spores had a harmful effect on both stand and yield.

DISCUSSION

The work here reported indicates that environmental conditions may have a marked influence upon the ability of the organisms carried in black point-infected seed to enter the seedling. The seed used became infected under conditions of high temperature in 1933. Temperatures were also high in 1934 under drought conditions during the development of the plant, but there was little black-point infection. Perhaps such conditions are adverse to the propagation of the disease from the seed. It would be of value to learn under what environmental conditions one might expect severe infestation and injury to result from black-point seed grown under field conditions.

Not much can yet be said as to the differences in stand from seed grown in the greenhouse and seed grown out of doors. The two lots of seed, healthy and greenhouse-grown, both had a germination of approximately 100 percent in the laboratory. It is possible that despite the good laboratory germination certain diseases carried by the seed had a lethal effect upon the seedling only when the seed germinated in soil. It would be a simple matter to test this point. Or perhaps the greenhouse-grown kernel had a better balanced reserve food supply which prevented the loss of the seedling under the stress of germination in the soil.

SUMMARY

As compared with crops from healthy seed, a crop raised from seed infected with black-point disease was not appreciably affected in yield or other characters, except for a slight difference in stand of seedlings.

The application of Ceresan to the diseased seed before planting had no apparent effect upon yield or other characters. As a matter of fact, the black point-infected seed treated with Ceresan yielded 4.3 bushels less than the untreated lot, which is a barely significant difference, but as healthy seed treated with Ceresan yielded 3.8 bushels more than the untreated seed, probably both of these deviations are accidental.

Application of bunt spores to the seed, reduced the yield in all cases to a significant degree, and the loss in yield of Ceres, a susceptible variety, was pronounced.

Yields of the composite hybrid selections from a Ceres × (Hope × Florence) cross were equal to those of the Ceres parent.

The seed grown in the greenhouse, which was exceptionally heavy in kernel weight, produced decidedly larger yields than the other seed tested. The larger yields from the greenhouse-grown seed were evidently due to larger initial and mature stands.

The interlocking type of experiment described in this paper was effective in reducing the standard error because of a relatively large number of degrees of freedom, comparatively small experimental differences thus showing significance.

THE INFLUENCE OF COMMERCIAL FERTILIZERS, POTASSIUM IODIDE, AND SOIL ACIDITY ON THE IODINE CONTENT OF CERTAIN VEGETABLES[1]

By WARREN B. MACK, *professor of vegetable gardening*, and EUGENE P. BRASHER, formerly *graduate assistant in vegetable gardening. Pennsylvania Agricultural Experiment Station*[2]

INTRODUCTION

Studies were begun at the Pennsylvania State College in 1931 to ascertain the influence of long-continued fertilizer treatments of field plots on the iodine content of vegetables grown on them, and to determine the extent to which the iodine content of certain vegetables was increased by an application of potassium iodide to field plots on which the acidity had been modified with sulphur or calcium hydroxide.

WORK OF OTHER INVESTIGATORS

Various aspects of iodine fertilization have been studied by other investigators, both with respect to the growth of plants and the yield of crops, and to the iodine content of the plants to which the iodine compounds were applied. A few studies also have been made to determine the influence of the iodine contained in certain fertilizers on the iodine content of vegetables; these studies, however, have dealt with applications of iodine-containing commercial fertilizers to the current crop, and not with long-continued use of these fertilizers on the same plots.

A number of investigators have reported that iodine compounds, either in nutrient solutions, pot cultures, or field plots, have improved the growth and yields of plants, under the conditions stated. Doerell (8)[3] found that 3.2 or 4.2 kg of iodine per hectare increased the yield of hops. He suggested that the iodine in commercial sodium nitrate or superphosphate, amounting to about 100 g per hectare, was beneficial to crops. Loew (22) reported increases in yield of both tops and roots of radishes fertilized with 0.5 and 0.05 g of potassium iodide to the plot of 20 m^2 as compared with unfertilized checks, the smaller application producing the largest yield. Malhotra (26) stated that moderate fertilization of soil with iodine—5.0 mg per kg of soil naturally containing 0.85 mg per kg—stimulated the growth of carrots somewhat. Mazé (28) found that to obtain full growth of maize in nutrient solutions of N, P, K, Ca, Mg, S, Fe, Mn, Zn, Si, and Ce from commercial chemical compounds in distilled water, it was necessary to add iodine, boron, aluminum, and fluorine. Scharrer and Schropp (32) observed a slight stimulation of growth of wheat growing in gravelly soil with nutrient solutions, when low concentrations of iodine compounds were used. Stoklasa (35, 37)

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improved the growth of sugar beets with 0.02 g of iodine as potassium iodide in 12 kg of soil per pot culture. Weiske (*40*) found that barley and spinach were stimulated by iodine compounds applied at about 5 to 10 times the rate contained in Chile saltpeter applications. Suzuki (*38*), and Suzuki and Aso (*39*) found that peas, oats, and radishes were stimulated by KI treatment.

Other investigators, however, have reported crop reduction or injury to plants as a result of iodine treatment. Beaumont and Karns (*2*) found a slight decrease in yield of turnips resulting from the application of KI at the rate of 2 kg per hectare. Cotton (*5*) observed injury to buckwheat from iodine as KI in nutrient solutions, in concentrations from 0.13 to 126.9 parts per million. Dafert and Brichta (*6*) found no differences in the growth of barley, mustard, and turnips resulting from the use of iodine in amounts equal to those present in the usual field applications of Chilean nitrate of soda. Doerell (*8*) noted a reduction in yield of hops from applications of 5.4 kg of KI per acre, though the difference was probably not significant.

Engels (*9*) reported no stimulative effect of either KI or $NaIO_3$ on sugar beets and mangels in field plots. Fellenberg (*13*) noted no effect on yield of mangels from 2.0 kg of KI per hectare. Hiltner and Bergold (*21*) reported similar results with 400 to 600 g of KI per acre, with sugar beets. Malhotra (*26*) found that the application of 32.0 mg of KI per kg of soil soon killed carrots growing on it. Scharrer and Schropp (31) observed injury to oats, wheat, rye, and barley, in decreasing order of damage, from the use of iodine salts and noted less unfavorable effects on clay soil than on muck soil. They reported the following order of damage for the different iodine salts: iodide $>$ iodate $>$ periodate. Weiske (*40*) showed that the amounts of iodine compounds present in commercial Chile saltpeter did not influence crops, though 5 to 10 times these amounts were usually injurious, except to barley and spinach. Wrangell (*41*) could find no differences in yield of potatoes, beets, oats, spinach, lettuce, pumpkins, red clover, sprouts, beans, or alfalfa with either 1.5 or 6.0 kg of iodine per hectare in KI or soot, in pot cultures or field plots. Wynd (*42*) found that iodine concentrations from 1 to 20 parts per million from KI, in Shive's nutrient solution, in all cases reduced the growth of tomato plants, and in concentrations above 5 parts per million caused injury to the leaves.

Many of the investigators named above and others have reported increases in the iodine content of plants to which iodine applications had been made. Beaumont and Karns (*2*), Conner (*4*), Densch, Steinfatt, and Günther (*7*), Fellenberg (*10, 13, 14*), Hercus and others (*17, 19*), Hiltner (*20*), Hiltner and Bergold (*21*), Malhotra (*26*), Maurer, Schropp, and Ducrue (*27*), Orr, Kelly, and Stuart (*29*), Pfeiffer and Courth (*30*), Scharrer and Schwaibold (*33*), Scharrer and Strobel (*34*), and Stoklasa (*36, 37*) all reported increases, in some instances tenfold, in the iodine content of plants receiving iodine applications as compared with that of control plants. The largest amount of iodine found in any plant tissues as the result of fertilization with iodine compounds was 1,419,000 and 1,184,000 parts per billion of dry weight of radish leaves and roots, respectively, reported by Pfeiffer and Courth (*30*). Only Wrangell (*41*) found no increase in iodine content of plants as a result of iodine fertilization.

The iodine contained in commercial fertilizers has been reported by several investigators as increasing the iodine content of the crop, while others have observed no such effect. Conner (4) stated that fish meal as a source of nitrogen increased the iodine content of vegetables. Hercus and others (18, 19) found that commercial fertilizers increased the iodine content of grasses, but ascribed this effect to improved growth rather than to the iodine content of the fertilizers. Analyses by Fellenberg (10, 11), McHargue, Roy, and Pelphrey (23), Hercus, Benson, and Carter (18), and Hercus and Roberts (19) showed that commercial fertilizers of mineral origin contain considerable quantities of iodine. With the exceptions noted above, however, the investigators did not carry out experiments to ascertain whether the iodine contained in the fertilizers actually increased the amount of iodine present in the crop as harvested.

The effect of acidity of the culture medium upon iodine absorption by plants was investigated to some extent by Fellenberg (15) and by Malhotra (26). The former studied iodine absorption on two very acid soils (pH 4.4 and 4.5) and on two moderately alkaline soils (pH 7.2 and 7.3). He found that carrots obtained more iodine, which was added as potassium iodine, from acid soils in pot cultures than from alkaline soils under similar conditions. He found, further, that acid soils liberated more iodine by catalytic action than did alkaline soils, and that the organic matter of the soil was active in fixing iodine in soils. Malhotra (26) found that iodine absorption by carrots in nutrient cultures was lowest at pH 9.0, next lowest at pH 4.0, and highest at pH 6.0; at pH 5.0, 7.0, and 8.0, absorption was intermediate. Densch, Steinfatt, and Günther (7) found that liming increased the iodine content considerably when no iodine was added to the soil, but had little effect with the maximum amount of added iodine.

METHODS

The analytical procedure in most of these studies was similar to that described by Frear (16), except for certain modifications in the combustion apparatus. According to this procedure, dried, ground samples of plant material are burned with commercial oxygen in a closed apparatus provided with chambers for precipitating smoke and for scrubbing volatile products of combustion; the ashes, precipitated smoke, and scrubbing solutions are collected, evaporated to dryness in excess of alkali, and the iodine compounds in them are extracted with alcohol. After the alcohol has been removed by evaporation, the iodine compounds are dissolved in water, oxidized, and estimated titrimetrically.

The final form of the combustion flask used in these studies was a wide-mouthed (53 mm) 1-liter pyrex Erlenmeyer flask, fitted with a rubber stopper through which were inserted an oxygen inlet tube of 5-mm bore, an outlet tube of 7-mm bore for gaseous products of combustion, and a thick-walled tube of 27-mm bore through which the sample was introduced for combustion, all of pyrex glass. The large tube was about 30 cm in length, and was inserted through the stopper so as to extend well into the body of the flask. The sample of dried, ground vegetable material was introduced through this tube in the way described by Frear. The oxygen inlet tube extended

to the end of the large tube inside the flask, and was bent toward the large tube at that point, so as to deliver oxygen directly to the point at which combustion took place (fig. 1).

The flask was supported by means of clamps in a horizontal position, so that ashes fell from the end of the sample as it was burned; the ashes were not subjected for any considerable length of time to the heat of the flame, as they were when the sample was burned in the vertical position employed by Frear.

The absorption apparatus included, in order, a water-jacketed condenser, a trap vessel for condensed liquids, and three Cottrell precipitators alternated with absorption towers containing glass beads moistened with saturated potassium carbonate solution.

Samples were prepared for analysis by slicing, drying at 70° C., grinding in a Wiley mill, and storing in glass-stoppered bottles. Before being ignited they were mixed with about 5 percent of their weight of powdered $CaCO_3$, which was found to improve the smoothness of burning of the sample. In other respects, the analytical procedure was similar to that followed by Frear (*16*), to whose
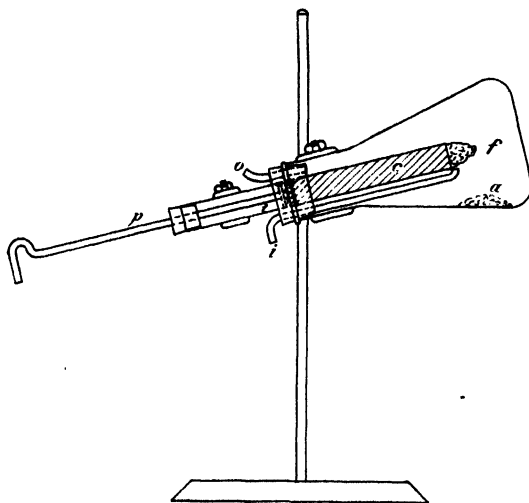
FIGURE 1.—Combustion apparatus for iodine analysis: *p*, Plunger for moving the cartridge of dried, ground sample, *c*, through the tube, *t*; *i*, oxygen inlet; and *o*, outlet for gaseous combustion products; combustion is carried on inside the flask, *f*; and ash, *a*, accumulates as it falls from the burning tip of the cartridge.

paper the reader is referred for details of the method.

The accuracy of the analytical method was determined by analysis of three different lots of vegetable material before and after the addition of known quantities of potassium iodide. Two lots of tomatoes and one of asparagus were used. Recoveries were made of from 95 to 99 percent of the quantities added. In asparagus, which was found to contain 108 micrograms of iodine, and to which 85 micrograms were added, the total amount recovered was 189 micrograms. With one lot of tomatoes, 378 micrograms of iodine were found after 360 micrograms had been added to the 20 micrograms originally determined in the sample itself; in the second lot, 358 micrograms were found after 360 micrograms has been added to a sample estimated to contain 15 micrograms of iodine.

Analyses were duplicated; if the results of the duplicates were not very closely in agreement, three or more analysis were made.

Tomatoes (*Lycopersicum esculentum* Mill.) sweet corn (*Zea saccharata* Sturt.), and potatoes (*Solanum tuberosum* L.) grown on certain of the plots in a fertilizer experiment with truck crops were analyzed, in an effort to find the relation between iodine content and the amount and kind of fertilizer used. The soil acidity of each plot

was measured to ascertain the relation, if any, between iodine content and soil acidity.

The fertilizers, which varied in the proportions of nitrate of soda, superphosphate, and muriate of potash, were similar in analysis to those that had been applied annually with a few exceptions, since 1917, to the plots from which the samples were taken. (A full description of the fertilizer experiment of which these plots are a part is contained in Pennsylvania Bulletin 210 (*25*). The fertilizers were applied broadcast and were harrowed in before the crops were planted. The particular lots used in the season in which samples were taken (1931) were Chilean nitrate of soda, 16 percent superphosphate, and muriate of potash. The amounts of these materials which were applied to the various plots are given in table 1.

Soil acidity in terms of pH was measured electrometrically, a potentiometer with a quinhydrone electrode being used.

TABLE 1.—*Iodine content of truck crops as influenced by fertilizer treatment and soil acidity*

POTATOES

Fertilizer treatment (pounds per acre)	Plot no.	pH of soil	Yield per acre	Iodine content on basis of —	
				Fresh weight	Dry weight
			Tons	*Parts per billion*	*Parts per billion*
625 superphosphate, 167 muriate of potash	1–8	6.60	4.4	46	180
	4–10	6.94	3.9	48	210
Average			4.2	47	195
600 nitrate of soda, 625 superphosphate, 167 muriate of potash	5–14	6.30	2.8	55	220
400 nitrate of soda, 167 muriate of potash	1–7	6.51	2.7	60	240
	4–11	6.42	3.9	50	200
Average			3.3	55	220
400 nitrate of soda, 938 superphosphate, 167 muriate of potash	5–10	6.80	5.7	41	190
400 nitrate of soda, 625 superphosphate	1–6	6.55	4.3	51	200
	4–12	6.66	2.9	40	150
Average			3.6	45.5	175
400 nitrate of soda, 625 superphosphate, 250 muriate of potash	2–12	6.55	4.5	93	410
	5–6	6.88	4.6	50	210
Average			4.6	71.5	310
Average				53.4	221

TOMATOES

625 superphosphate, 167 muriate of potash	1–8	6.60	13.4	16	230
	4–10	6.73	10.2	8	240
Average			11.8	12	235
600 nitrate of soda, 625 superphosphate, 167 muriate of potash	5–14	6.30	14.7	8	170
400 nitrate of soda, 167 muriate of potash	1–7	7.36	11.1	19	250
	4–11	6.77	8.1	12	220
Average			9.6	15.5	235
400 nitrate of soda, 938 superphosphate, 167 muriate of potash	5–10	6.59	13.2	7	150

TABLE 1.—*Iodine content of truck crops as influenced by fertilizer treatment and soil acidity*—Continued

TOMATOES—Continued

Fertilizer treatment (pounds per acre)	Plot no.	pH of soil	Yield per acre	Iodine content on basis of—	
				Fresh weight	Dry weight
			Tons	*Parts per billion*	*Parts per billion*
400 nitrate of soda, 625 superphosphate	{ 1–6	7.52	17.1	14	300
	{ 4–12	6.87	13.8	17	370
Average..			15.5	15.5	335
400 nitrate of soda, 625 superphosphate, 250 muriate of potash.	{ 2–12	6.28	17.5	8	160
	{ 5–6	6.38	15.8	13	320
Average..			16.7	10.5	240
Average..				12.2	241

SWEET CORN

625 superphosphate, 167 muriate of potash..................	{ 1–8	6.57	2.7	24	90
	{ 4–10	5.96	3.9	25	100
Average..			3.3	24.5	95
600 nitrate of soda, 625 superphosphate, 167 muriate of potash..	5–14	6.67	3.8	73	250
400 nitrate of soda, 167 muriate of potash....................	{ 1–7	6.59	2.4	37	140
	{ 4–11	6.09	2.4	24	120
Average..			2.4	30.5	130
400 nitrate of soda, 938 superphosphate, 167 muriate of potash..	5–10	6.74	4.8	50	190
400 nitrate of soda, 625 superphosphate.....................	{ 1–6	5.85	3.1	33	120
	{ 4–12	6.68	3.8	23	90
Average..			3.5	28	105
400 nitrate of soda, 625 superphosphate, 250 muriate of potash..	{ 2–12	6.73	3.2	30	100
	{ 5–6	6.43	4.7	33	120
Average			4.0	31.5	110
Average..				35.2	132

The plots are listed in table 1 in such a way as to show the effect of the presence or absence of each of the critical plant-food elements, nitrogen, phosphorus, and potassium. The first two plots in the table, plots 1–8 and 4–10, received phosphorus and potassium but no nitrogen; the third plot, 5–14, received the same amounts of phosphorus and potassium as the first two plots, together with a fairly heavy application of nitrogen. The fourth and fifth plots, 1–7 and 4–11, received nitrogen and potassium, but no phosphorus; the sixth plot, 5–10, received the same amounts of nitrogen and potassium as plots 1–7 and 4–11, together with a relatively heavy application of phosphorus. Similarly, plots 1–6 and 4–12 received nitrogen and phosphorus, without potassium, and plots 2–12 and 5–6 received the same amounts of nitrogen and phosphorus, together with a heavy application of potassium. The pH of most of the plots varied but little; the maximum variation for any crop was between 6.28 and 7.52, for tomatoes.

INFLUENCE OF FERTILIZER TREATMENT AND SOIL ACIDITY ON THE IODINE CONTENT OF POTATOES, TOMATOES, AND SWEET CORN

The variation in iodine content of potatoes from the different plots (table 1) was not great, with the exception of those from plot 2–12, which received a generous application of potassium.   Potatoes from the second plot receiving the same fertilizer treatment (plot 5–6) did not differ widely in iodine content from those from the other plots.   Obviously, there is no relation between either the fertilizer treatment, the acidity of the soil, or the size of the crop, and the iodine content of potatoes grown on the experimental plots.*   The same is true for tomatoes, although the iodine content of this crop was somewhat more variable among the different plots than was the case with potatoes.   The iodine content of sweet corn apparently was increased by nitrate of soda on plot 5–14, as compared with plots 1–8 and 4–10; all other plots, each of which received two-thirds as much nitrate of soda as did plot 5–14, differed but little with respect to the iodine content of the sweet corn grown on them, from the plots without nitrogenous fertilizers.

The average iodine content of sweet corn from all plots, 132 parts per billion of the dry samples, was considerably below that of the tomatoes, 241 parts per billion, and that of the potatoes, 221 parts per billion.   The difference between the tomatoes and potatoes in this respect is not significant.

INFLUENCE OF IODINE FERTILIZATION ON THE IODINE CONTENT AND YIELD OF BEANS AND TURNIPS

Studies were carried out in 1931 with beans (*Phaseolus vulgaris* L.) and turnips (*Brassica rapa* L.) to ascertain whether any considerable increase in iodine content might be effected economically by applications of an iodine compound to field plots.   Soil acidity was introduced as an experimental variable, by modifying the acidity through additions of hydrated lime or sulphur.

Five plots, each 20 by 24 feet, were marked out on July 20, and materials were applied as follows:

Plot 1—44.0 pounds of hydrated lime (2 tons per acre).
Plot 2—22.0 pounds of hydrated lime (1 ton per acre).
Plot 3—Check.
Plot 4—66.0 pounds of sulphur (3 tons per acre).
Plot 5—110.0 pounds of sulphur (5 tons per acre).

These materials were broadcast and harrowed into the soil.   Five rows each of Sure Crop Stringless Wax beans and Purple Top White Globe turnips were seeded on the same day, the beans in rows 30 inches apart, and the turnips in rows 18 inches apart, parallel to the longer side of the plots.

The hydrogen-ion concentration was measured electrometrically from time to time, to ascertain the progress of the change of acidity brought about by the materials applied.   The results of these measurements are shown in table 2.   The effects of the hydrated lime were immediate, while those of sulphur were not apparent before the second measurement, more than 2 weeks after the sulphur was applied.

TABLE 2.—*pH of plots on four dates during the iodine fertilization experiment*

Plot no.	pH of plots			
	July 27	Aug. 6	Aug. 18	Sept. 22
1	8. 33	8. 22	8. 34	8. 23
2	8. 25	7. 65	8. 33	8. 16
3	7. 73	7. 56	7. 61	7. 60
4	7. 74	6. 80	6. 12	5. 85
5	7. 73	6. 50	5. 92	4. 95

On August 21 the corresponding half of each plot, including half of each row of turnips and of beans, was fertilized with 12.984 g of potassium iodide, or at the rate of 2.356 kg to the acre. This was distributed by dissolving it in water and sprinkling it evenly upon the surface of the soil, between the rows of beans and turnips, the former of which were beginning to blossom by this time. After the solution had been distributed, the plants were sprinkled thoroughly with water, so that none of the solution would remain on the portions of the plants above ground. The iodine added to the upper 6 inches of soil in this application amounted to approximately 2.0 mg, or 2,000 micrograms, per kilogram, which is about one-half of the total iodine content of certain agricultural soils, as reported by Chatin (*3*), Fellenberg (*12*), Andrew (*1*), and McHargue, Young, and Roy (*24*). Each square foot of soil surface received 0.0413 g of iodine. The application was delayed until this time in order that marked differences in soil acidity might be brought about before the iodine was applied.

The effects of soil acidity and of iodine became evident within 2 weeks after the potassium iodide was applied. Both the beans and the turnips were noticeably more vigorous on the more alkaline lots, and were much less vigorous on the more acid plots. The turnips were especially low in vigor on the plots to which sulphur had been added. Probably this was due, in part, to the unfavorable soil acidity, but to a considerable extent it was attributable to aphid infestation. These infestations were severe only on the plots that had received sulphur applications. The effects of the potassium iodide on the two crops were opposite in character: the turnips were noticeably improved in growth, while the beans were injured, as was shown by the dropping of the lower leaves and the yellowing of the remaining ones, and a general stunting of growth.

The crops were harvested when they reached commercial maturity and their fresh weights recorded. The weights of the green bean pods also were recorded, and the entire harvest from each plot was prepared for iodine analysis. The turnips were weighed and 5-kg samples, made up of entire plants, were taken for analysis. In harvesting and sampling for analysis, a 2-foot strip between the plots, and between the iodine fertilized and unfertilized halves, was excluded. The harvested portions of the half plots, therefore, were 10 by 20 feet, of which 10 by 12.5 feet were occupied by beans, and 10 by 7.5 feet by turnips.

The yields and the iodine content in parts per billion of dry and of fresh weight of the crops from each plot are recorded in table 3. In this table, the halves of the plots to which potassium iodide was applied are designated by the letter A, while the unfertilized halves are designated by the letter B.

TABLE 3.—*Effect of soil acidity and potassium iodide fertilization with KI on the yield and iodine content of beans and turnips*

Plot no.[1]	pH of soil		Beans			Turnips		
	When KI was applied	When crop was harvested	Yield per plot	Iodine content on basis of—		Yield per plot	Iodine content on basis of—	
				Fresh weight	Dry weight		Fresh weight	Dry weight
			Kilograms	*Parts per billion*	*Parts per billion*	*Kilograms*	*Parts per billion*	*Parts per billion*
1A	8.34	8.23	6.75	73	730	37.55	9,496	94,960
1B	8.34	8.23	8.20	26	280	32.12	79	740
2A	8.33	8.16	3.85	84	1,170	27.13	5,692	51,740
2B	8.33	8.16	6.23	57	510	25.99	85	850
3A	7.61	7.60	2.33	71	750	22.50	2,044	19,540
3B	7.61	7.60	4.68	26	260	21.64	180	1,590
4A	6.12	5.85	2.43	56	570	9.89	2,607	24,140
4B	6.12	5.85	3.58	45	490	7.71	209	2,080
5A	5.92	4.95	1.21	122	1,410	7.63	2,148	21,140
5B	5.92	4.95	4.24	54	630	7.03	191	1,910

[1] The halves of plots to which KI was added are designated by the letter A, the unfertilized halves by B.

Table 3 shows that the yield of beans was reduced in every case by the addition of potassium iodide to the soil; the yield of turnips, on the other hand, was increased by iodine fertilization in every case. In general, the yields of both beans and turnips decreased rapidly as the acidity increased.

In turnips, which were stimulated in growth by fertilization with potassium iodide, the iodine content was enormously increased by this means. The increase ranged from a little more than tenfold on plots 3, 4, and 5, to about 120-fold on plot 1, on the basis of fresh weights. On the other hand, the iodine content of the beans, which were injured by potassium iodide, was increased to less than three-fold in all cases. The maximum increases in iodine content of turnips occurred on the most alkaline plots; the increases in iodine content of the beans evidently were not related to the acidity of the soil. On plots treated with potassium iodide, the iodine content of turnips was greater the higher the pH of the soil, except on plot 3; on plots without potassium iodide applications, on the other hand, the iodine content was greater the lower the pH, except on plot 5.

Turnips obtained far greater quantities of iodine from the soil to which no iodine was added than did beans. The fact that the entire plants of turnips were analyzed, however, while only the green pods were used for the measurements of iodine content of beans, should be considered.

On the basis of the yield and iodine content of turnips on plot 1A, the recovery of the added iodine by the turnips on this plot was 0.3536 g, or 11.4 percent of the amount added.

In view of the small recovery of added iodine, the plots were planted in 1932 in the same way as in 1931, but without applying any additional sulphur, hydrated lime, or potassium iodide, in order to ascertain whether any residual effect of the iodine applied in 1931 could be detected. No consistent effect could be observed in the growth and yield of either beans or turnips, both of which produced considerably smaller yields than in 1931. A number of these samples have been analyzed, and no residual effects of the potassium iodide applied in the previous season have been found.

SUMMARY

No relation was observed between the iodine content of potatoes, tomatoes, and sweet corn grown on field plots in a long-continued fertilizer experiment, and either the fertilizer treatment, the soil acidity, or the yield of the crop. The fertilizers used in the experiment were Chilean nitrate of soda, superphosphate, and muriate of potash.

An application of potassium iodide at the rate of 2.356 kg to the acre, supplying approximately 2.0 mg of iodine to the kilogram of soil in the upper 6 inches, increased the iodine content of green beans considerably as compared with that of beans similarly grown but without iodine fertilization, but it injured the plants and reduced the yields markedly. Similar applications of potassium iodide increased the yields of turnips somewhat, and increased the iodine content of the turnip plants from tenfold to 120-fold, on different plots. The greatest increases in iodine content of turnips occurred on plots made alkaline with hydrated lime, and the smallest increases on plots made acid with sulphur or unmodified in acidity.

The iodine content of turnips from plots not fertilized with potassium iodide was generally greater the more acid the soil; the iodine content of beans not treated with potassium iodide, however, was not influenced consistently by soil acidity.

The maximum recovery of added iodine by any turnip plants in the experiments was 11.4 percent. Analyses of plants grown on the same plots in the second season, without additional iodine, indicated that there was no residual effect of the iodine application.

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A CYTOLOGICAL STUDY OF ERYSIPHE POLYGONI ON DELPHINIUM [1]

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INTRODUCTION

Recent cytologic and genetic studies of the higher fungi have resulted in a marked alteration of the point of view as to the nature of sex, the process of sexual reproduction, the alternation of generations, and the genetics of fungi. The cytology of the mildews has been much studied in the past, but it has seemed worth while to reexamine the mildews in the light of recent advances in other ascomycetes and in the basidiomycetes.

MATERIAL AND METHODS

Material for this study was obtained from a *Delphinium* plant growing out of doors in a damp, partly shaded place. The plant was growing rapidly and was covered with a luxuriant growth of mildew bearing thousands of perithecia in all stages of development. The material was fixed in Flemming's weak solution and in Flemming's weak solution diluted to two-thirds and to one-half strength, respectively. The fixed material was washed and dehydrated and then embedded in paraffin, sectioned 12μ thick, and stained with Heidenhain's iron haematoxylin with a counter stain of Congo red.

INVESTIGATIONS

MYCELIUM

Plate 1, *A*, represents a detail of the young, superficial, vegetative mycelium of *Erysiphe polygoni* DC. The hyphae consist of long, straight cells, fairly uniform in diameter except at the formation of a haustorium, a vegetative branch, or a conidiophore. No detailed study has been made of the formation of haustoria or the development of the conidiophores.

Vegetative cells of young mycelium are uninucleate. The vegetative nucleus may be located at any point within the cell. It varies greatly in shape (pl. 1, *B*, *C*, *D*, *E*, *F*, *G*), but always contains abundant

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chromatin, a large protruding "nucleole" at one end, and usually one or two dark-staining smaller granules located elsewhere on the nuclear membrane.

A comparison of the drawings shown in plate 1, *B* to *G*, suggests that the nucleus possesses the power of locomotion. The shape of the nucleus varies greatly (*B*, *C*, *D*) and not infrequently (*E*, *F*, *G*) the "nucleole" is prolonged into a slender rod terminating in a small knob which projects out into the cytoplasm. It may be that this nucleolar apparatus is a mechanism for locomotion. Details of the process have not been worked out.

The question arises as to whether a nucleus can pass through a septum from one cell of a hypha to another. Cytoplasmic connection through a septum between adjoining cells is probably always present. These connections are seen most clearly in cells that are slightly plasmolyzed (pl. 1, *H*, *a*), but in turgid cells also the configuration of the cytoplasm often suggests direct streaming of cytoplasm through the center of a septum. Plate 1, *I*, shows an immature conidium. One both sides of each septum, strands of cytoplasm converge upon the center of the septum. If minute openings in septa are of regular occurrence it would seem possible for a nucleus also to pass through. But on the supposition that nuclei migrate through the mycelium one would expect to find irregularities in the number of nuclei in the cells. A count of 100 vegetative cells gives 97 cells with 1 nucleus and 3 cells with 2 nuclei. These three binucleate cells are easily accounted for as incidental to growth and the formation of new cells. There is no evidence, then, that in purely vegetative mildew mycelium any nucleus leaves the cell of its birth.

DEVELOPMENT OF THE PERITHECIUM

Delphinium mildew has not been proved to be heterothallic, but heterothallism is now known to exist in the sunflower mildew (*59*)[3] and the early stages of reproduction in the two mildews are similar.[4]

The early stages in the sexual reproduction of delphinium mildew have been studied, and in no case has it been found that the two hyphae concerned originated on the same individual. In sectioned material, of course, the mycelium is cut, and it is rarely possible to trace a hypha far; but, if the species were homothallic, it is unlikely that among the hundreds of cases studied a few would not have been found in which the connection between the two hyphae could be proved. It is probable, although not proved, that there are (+) and (−) strains here, as in sunflower mildew, and that only when the two meet does reproduction take place.

In a mass culture of young vegetative mycelium (supposedly containing both (+) and (−) strains) the hyphae manifest no attrac-

[3] Reference is made by number (italic) to Literature Cited, p. 816.
[4] Unpublished data.

EXPLANATORY LEGEND FOR PLATE 1

A.—Young vegetative mycelium. Mass culture. × 230.
B, *C*, *D.*—Nuclei of vegetative mycelium showing different shapes and positions. × 2,250.
E, *F*, *G.*—Nuclei of vegetative mycelium showing prolongation of nucleole into a slender rod capped by a small knob. × 2,250.
H.—Slightly plasmolyzed mycelial cell showing (at *a*) cytoplasmic connection from one cell to the other through the septum. × 2,250.
I.—Immature conidium showing strands of cytoplasm converging on the center of the septa. × 1,400.
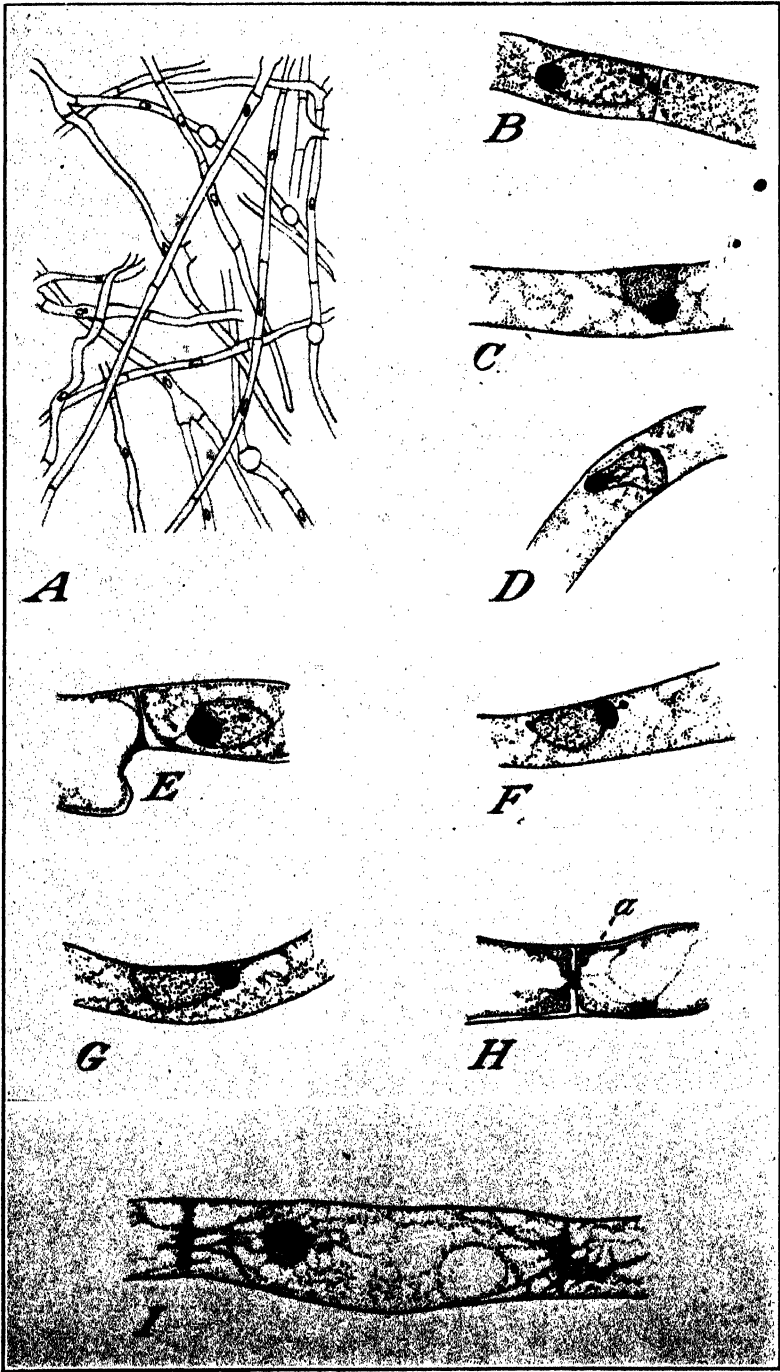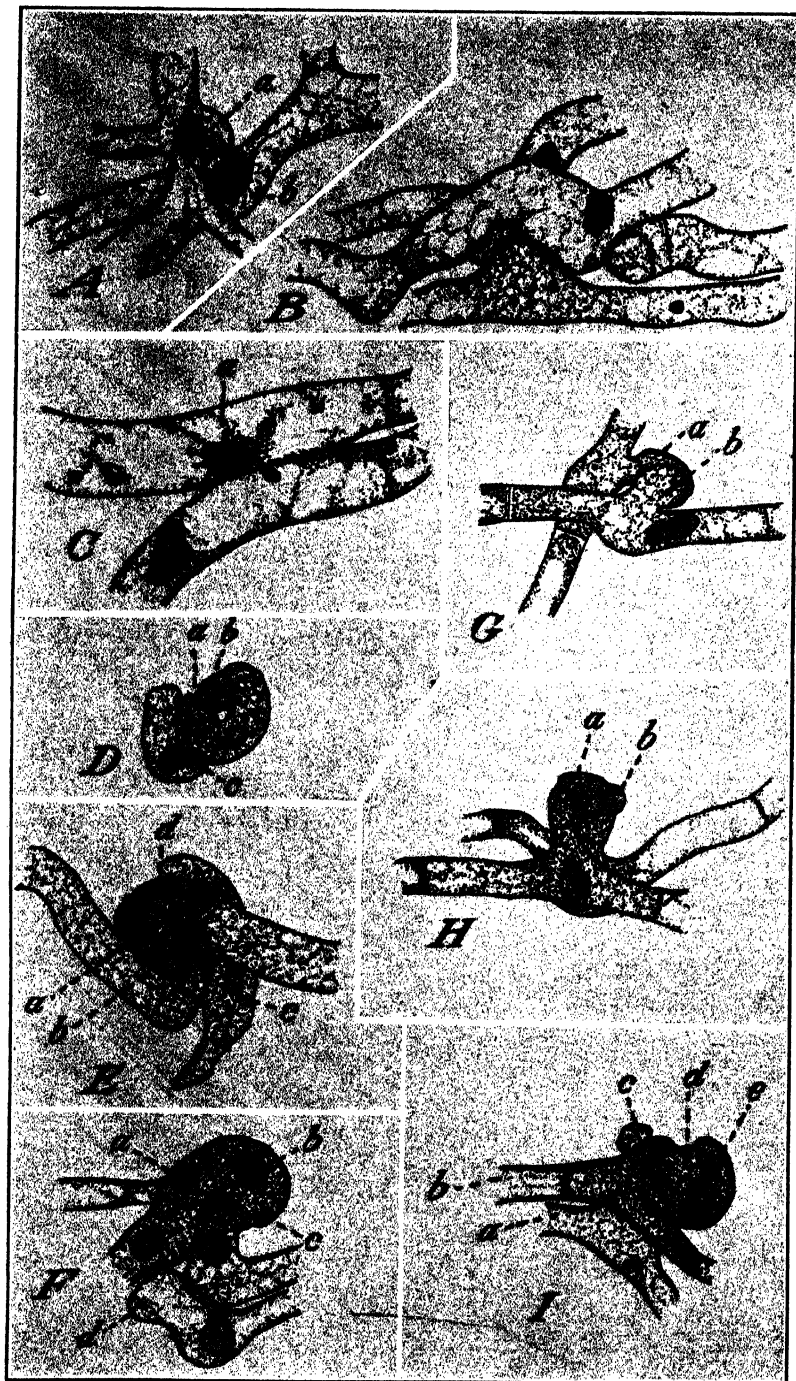
tion for each other. Contacts between hyphae occur only when the mycelial mat is so dense that contacts are unavoidable. When an accidental contact occurs (pl. 1, *A*) the two hyphae continue their growth with unaltered size and direction.

Quite otherwise is their behavior a day or two later at the inception of reproductive activities. Contiguous hyphae, supposedly (+) and (−), swell toward each other, forming an extensive surface of contact. The first relations between the two present great diversity in appearance. The initial surface of contact between two hyphae may be at the growing tips or farther back, or one growing tip may make contact with an older portion of a second hypha.

Usually only two hyphae are concerned at the start, as in plate 2, *A*. Here one hypha is swelling at *b* and the other is beginning to push out a branch along the line of contact at *a*. Occasionally more than two are involved. Plate 2, *B*, represents an extreme case in which four hyphae have swollen and taken on a sinuous growth, with extensive areas of contact with each other. Rarely the initial contact between two hyphae serves to stimulate the passage of a nucleus from one to the other, as in *C*, *a*.

When the initial contact is between the growing tips of two hyphae (pl. 2, *D*, *a*, *b*), both tips thicken and curl up. The cells are usually uninucleate, the cytoplasm is dense, and dark-staining granules (*D*, *c*), probably food, appear in the cytoplasm. In the further development of this type (*E*) one hypha, *a*, coils around the other, *b*. In this case, a third hypha, *c*, has become associated with them. At *d* is a dark-stained body in the act of passing through a septum. Whether this is a food granule or a much-condensed nucleus is not clear. In plate 2, *F*, the terminal cells, *a*, *b*, of the two hyphae have established communication, and cytoplasm is passing through from *a* to *b* at *c*. Here, also, a third hypha, *d*, is affiliated.

In the majority of cases the initial surface of contact between two hyphae is formed at some distance back from the growing tips. In plate 2, *G*, the two hyphae grew past each other at an angle and thickened at the area of contact, and each is pushing out a short, thick branch. The two young branches (*G*, *a*, *b*) are closely associated and are equal in size and similar in shape. In plate 2, *H*, the branches, *a*, *b*, are larger and somewhat coiled about each other. In this case there is a distinct difference between the two. One, *a*, is shorter, thicker, and less twisted than the other, *b*. In plate 2, *I*, a slightly later stage, the difference is more marked. The hypha *a* has formed a short, thick, curved branch, *d*, and the hypha *b* has produced two slender hyphae, *c* and *e*, which are wrapped around the first. Plate 3, *A*, shows a still later stage. The two hyphae with their branches

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EXPLANATORY LEGEND FOR PLATE 2

A.—Beginning of sexual reproduction. Two hyphae in contact, with swellings at *a* and *b*. × 1,400.
B.—Four swollen hyphae in sinuous growth, with extensive areas of contact with each other. × 1,400.
C.—Two mycelial hyphae in contact. A nucleus, *a*, is passing from one hypha to the other. × 2,250.
D.—Area of contact between curled up growing tips of hyphae, *a* and *b*. Granule, possibly food, at *c*. × 1,400.
E.—Growing tips of two hyphae, *a* and *b*, curled about each other. Third hypha at *c*. At *d* a dark-stained body is passing through a septum. × 1,400.
F.—Curved growing tips of two hyphae, *a*, *b*, in communication at *c*. Third associated hypha at *d*. × 1,400.
G.—Two hyphae in contact pushing out branches, *a*, *b*. × 1,400.
H.—Two hyphae with young branches, *a*, *b*, twisted about each other. × 1,400.
I.—Hypha *a* has formed heavy branch, *d*, and hypha *b* has pushed out slender branches *c*, *e*, wrapped about *d*. × 1,400.

are shown in their natural relation in A_1, while in A_2 and A_3 the two are drawn separately. One, A_2, has produced a large, heavy, uninucleate cell with dense cytoplasm, accompanied by a much slenderer branch, while the other A_3, has formed a hypha whose slender branches clasp the large cell of A_2. Two cells in A_3 are binucleate.

In this case (pl. 3, A) it would be easy to take for granted that A_2 is the female branch and A_3 the male branch. In some of the other cases presented here, however, it would be difficult to assign sex.

In plate 3, B, is shown an irregular case. Three hyphae, a, b, c, are involved. The hypha af has formed a thickened branch, which passes under b and emerges at d. The hypha b has formed a large, thick, irregular branch, e, which has extensive areas of contact with c and with both parts of a.

In a few cases the growing tip of one hypha makes contact with an older portion of a second hypha. In plate 3, C, a broad surface of contact has formed between the swollen second cell of hypha a and the swollen tip of hypha b and an opening has formed between the two cells at c. Here it would be difficult to decide which is male and which is female. In plate 3, D, the tip of hypha a met the hypha bc at right angles. The latter pushed out a short, thick branch, e; the growing tip of hypha a thickened and twisted about it; and a broad opening between the two has formed at the apex, d. In plate 3, E, also, the growing tip of one hypha, a, crossed an older hypha, b, then stopped growing and formed a thickened binucleate terminal cell, c. Meanwhile the older hypha, b, thickened and is pushing out slender branches to meet c. In plate 3, F, the tip of hypha a met b, then turned and grew parallel to b, while slender branches from b have grown out to a and partly enclosed it.

In the majority of cases the fusion is between terminal cells of reproductive branches. There are exceptions. In plate 2, C, already described, a nucleus is passing between two older cells, neither of which is terminal. In plate 3, C, also described, the terminal cell of one hypha fused with the second cell of the other. In the example shown in plate 3, G, a nucleus is moving from hypha a into the third cell of hypha b. The nucleole leads in the advance of the nucleus. In plate 3, H, the nucleus, c, is moving from an intercalary cell of hypha b into a. As a portion of a was removed in sectioning, its position in its own hypha is unknown.

In the further development of the perithecium the narrow initial opening between the two fusing cells seen in plate 2, C, F, and plate 3, C, D, G, H, broadens rapidly. In plate 4, A, the fusion between the large cell, a, and the smaller cell, b, is complete. Two nuclei of unequal size are seen in the large cell (a). The fusion cells shown in

EXPLANATORY LEGEND FOR PLATE 3

A.—Two reproductive branches drawn together in their natural relation in A_1 and drawn separately in A_1 and A_3.

B.—Hypha af formed heavy branch passing under b and emerging at d. Hypha b formed heavy branch, e, which has broad areas of contact with af, ad, and c.

C.—Enlarged tip of hypha b in communication at c with enlarged second cell of hypha a.

D.—Hypha bc formed heavy branch e, which is in communication at d with enlarged tip of hypha a.

E.—Binucleate terminal cell, c, of hypha a, stopped growing and thickened. Slender branches from hypha b are pushing out to meet c.

F.—Hypha a met b, then turned and grew parallel to it. Hypha b is pushing out branches to a.

G.—Nucleus from hypha a moving into third cell of hypha b.

H.—Nucleus, c, from hypha b moving into hypha a. All × 1,400.
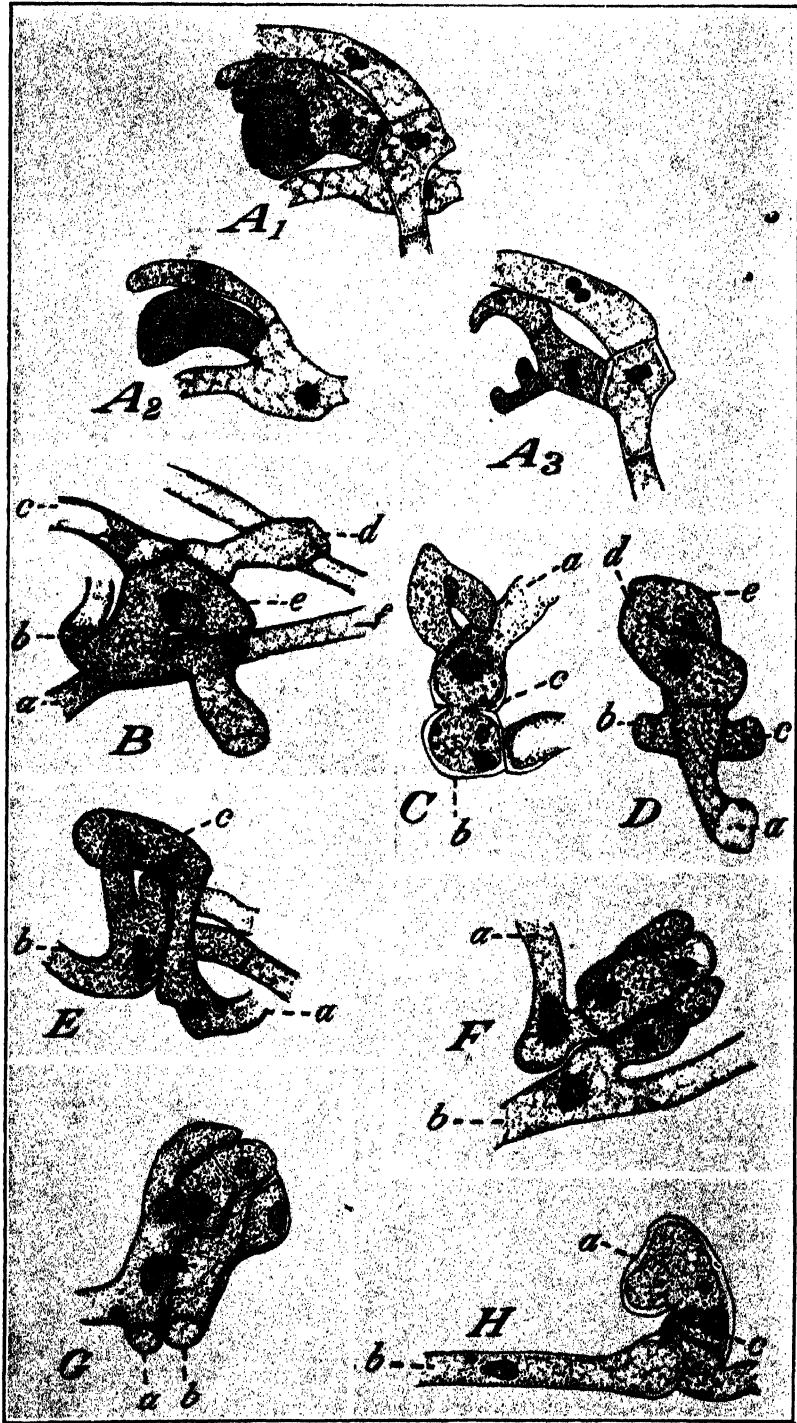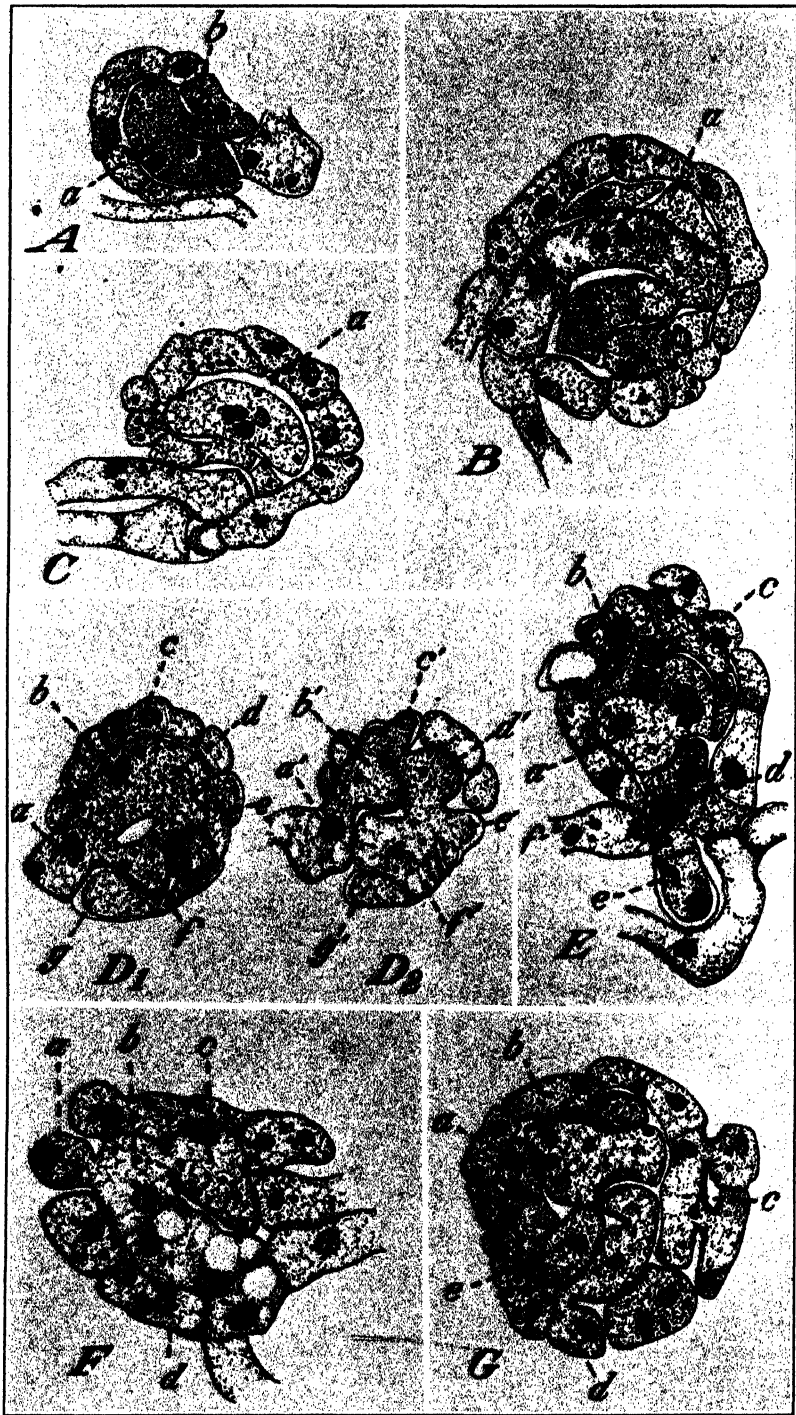
plate 4, *B* and *C*, are similar except that in *C* there are three nuclei in the center at *a*. No indication of a nuclear fusion at this time has been seen. During this time a layer of enveloping hyphae (the beginning of which has been seen in pl. 3, *A* and *F*) has formed, enclosing the central cell.

At first sight, plate 4, *A*, *B*, *C*, would seem to be clear evidence that two well-defined cells, an antheridium and an oogonium, fuse, and that their nuclei become associated. But drawing a perithecium of this age at a carefully chosen focus, as was done in *A*, *B*, *C*, can give a false impression of the situation.

By focusing up and down through a section (cut 12μ thick), it can be determined that many of the cells of these little perithecia are in open communication with one another. Plate 4, D_1 and D_2, illustrate this. The plane represented in D_1 is similar to that in *A* and *C*, but by focusing down, the picture changes gradually to D_2. Careful focusing up and down shows that cell *a* is continuous with *a'*, *b* with *b'*, *c* with *c'*, etc., and the fact emerges that a large portion of this little perithecium, including at least a part of its peripheral layer, forms a continuous passageway, and that the combination is multinucleate. A similar study of the perithecia drawn in *A*, *B*, *C* shows that in them also there are continuous mazes of communicating chambers.

As the point is of theoretic interest, it was studied further. In plate 4, *E*, the large trinucleate central cell, *a*, consists of two nearly equal lobes. Note the branching multinucleate maze, *b*, and the successive constrictions in *c*. The stalk cells, *d*, *e*, and *f*, are binucleate.

In the great majority of perithecia of this age it is still possible to locate the initial point of fusion. In the perithecium drawn in plate 4, *F*, however, it is uncertain whether the initial fusion took place in the three-lobed series *a*, *b*, *c*, or in the binucleate central cell, *d*. Perhaps even here the fusion could have been located with certainty if the perithecium had been cut at some other angle.

In plate 4, *G*, is drawn a transverse section through a perithecium. At the right, an added peripheral layer of the perithecium is forming by the proliferation of the cell *c*. A continuous passageway can be traced through the whole diameter of this perithecium from *b* to *d*. Another shorter maze runs from *a* to *e*. The question arises as to how much of one of these mazes should be considered one cell. As mentioned earlier (p. 802), it is probable that in vegetative mycelium minute openings in the septum between one cell and the next are regularly present. It may be that in young perithecia these openings between cells are much larger and more conspicuous. If this is true the segment of a maze between each constriction and the next would be one cell.

EXPLANATORY LEGEND FOR PLATE 4

A.—Young perithecium. Cells, *a* and *b*, fully fused.
B.—Fusion cell, *a*, with two nuclei.
C.—Fusion cell, *a*, with three nuclei.
D.—D_1 and D_2 show two different levels in the same section of a perithecium, in which *a* is continuous with *a'*, *b* with *b'*, etc.
E.—Perithecium with trinucleate central cell, *a*, short mazes at *b* and *c*, and binucleate stalk cells, *d*, *e*, *f*.
F.—Perithecium with short maze, *a*, *b*, *c*, and binucleate cell *d*.
G.—Transverse section of perithecium with maze from *b* to *d*, and another from *a* to *e*. New outer layer of perithecium forming from *c*. All × 1,400.

In plate 5, *A*, is shown a longitudinal section of a perithecium with its mycelial connections. Several hyphae contributed to the formation of this perithecium. The maze of communicating cells is so intricate and complete that one gains the impression that more than one fusion was necessary to produce it. In cases like this it would be impossible to determine how much of such a maze formed as a result of growth and cell divisions with incomplete closure of the septa, and how much, if any, arose through subsidiary fusions.

When two cells and the opening between them lie in one horizontal plane, the fact is easily determined. When a cell connects with another above or below it, it is much more difficult to be sure. It is theoretically possible that, in some cases at least, if all the connections in a small perithecium could be determined the whole perithecium would be found to consist of one intricate communicating system. This has not been proved. Moreover, the extent of the intercommunication seems to vary in different perithecia. The perithecia in plate 5, *B* and *C*, for example, show the minimum of connections. A few connections are to be seen here, and others occur in these same perithecia in planes above and below the one drawn; but it is highly improbable, in these perithecia at least, that all the cells are connected.

During this development there is a rapid increase in the number of nuclei. The increase is most conspicuous in the fusion cell. Directly after fusion the central cell contains two or, at most, three nuclei (pl. 4, *A*, *a*; *B*, *a*; *C*, *a*; *E*, *a*). Soon after this it contains four or five (pl. 5, *B*, *a*), then six or eight (pl. 5, *C*, *a*; *D*, *a*; *E*, *a*; *F*), and still later a dozen or more (pl. 5, *G*).

It is not the fusion cell only that shows this increase in the number of nuclei. A survey of plates 4 and 5 shows that throughout the young perithecium the number of nuclei is on the increase. The question arises as to the source of these nuclei. Do they form by nuclear divisions in the perithecium or do nuclei migrate in from the mycelium? The nuclear content of stalk cells connecting the perithecium to the mycelium is most variable. In plate 5, *A*, the stalk cell at *a* contains no nuclei; the one at *b* contains two. The mycelial connection at plate 5, *D*, *b*, contains three nuclei, and the one at plate 5, *E*, *b*, contains four. Four and five nuclei are common in the stalk cells in plate 6, *B*, and plate 8, *C*.

If surplus nuclei are forming in the mycelium and moving into the perithecium, the nuclear content of mycelial cells would be irregular. As stated earlier, a count of 100 cells in young vegetative mycelium shows that 97 are uninucleate and 3 are binucleate. A similar count in mycelium bearing numerous small perithecia shows that of 100 mycelial cells 88 are uninucleate and 12 binucleate. There may be a slight justification here for the supposition that extra nuclei form in the mycelium and migrate to the perithecia, but if this occurs it is probably a minor factor. Moreover, only once or twice in this older

EXPLANATORY LEGEND FOR PLATE 5

A.—Longitudinal section of perithecium showing mazes. Mycelial stalk cell at *a* with no nucleus and one at *b* with two nuclei.
B and *C*.—Perithecia with few short mazes. Multinucleate central cell at *a*.
D.—Larger perithecium with multinucleate central cell, *a*, and trinucleate stalk cell, *b*.
E.—Perithecium with multinucleate decadent central cell, *a*, and multinucleate stalk cell, *b*.
F and *G*.—Multinucleate central cells. All × 1,400.
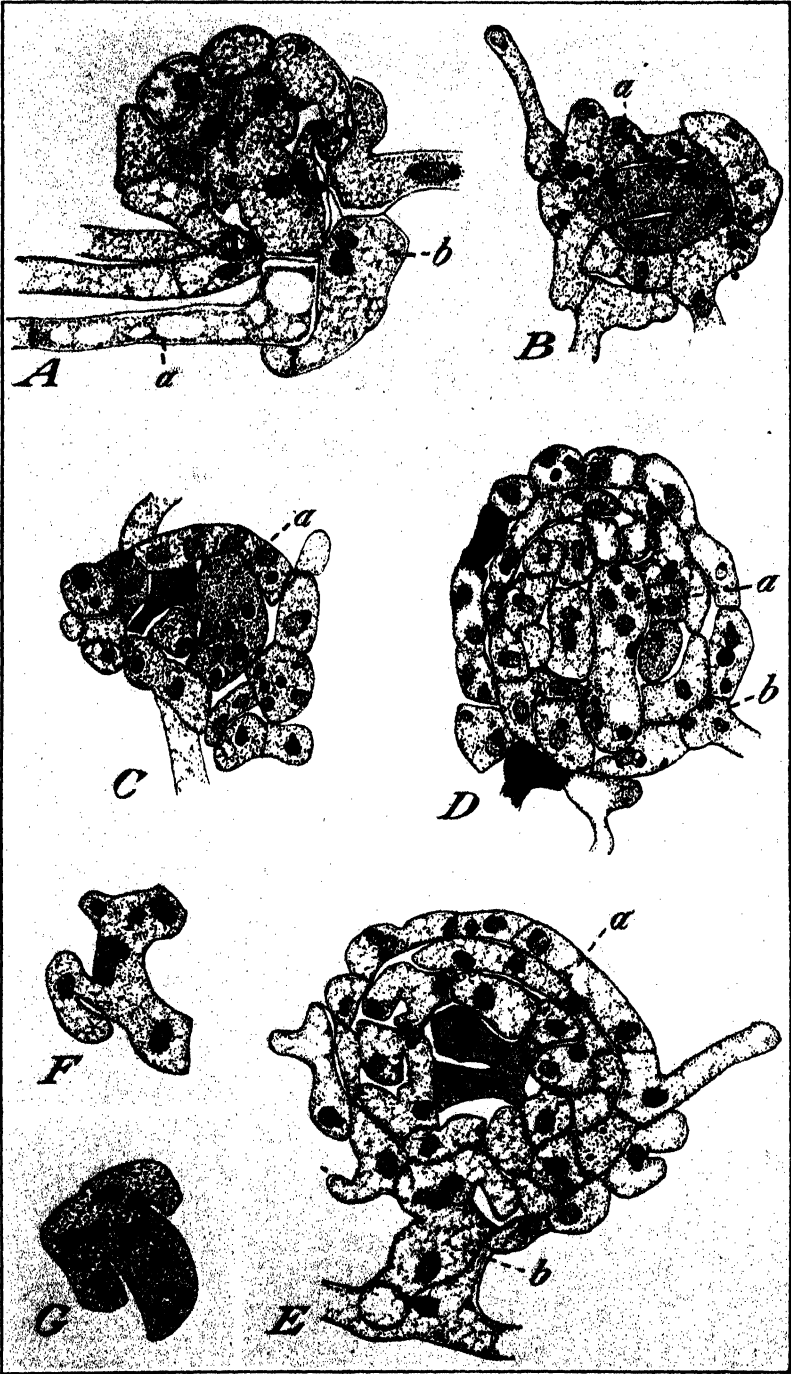
PLATE 5
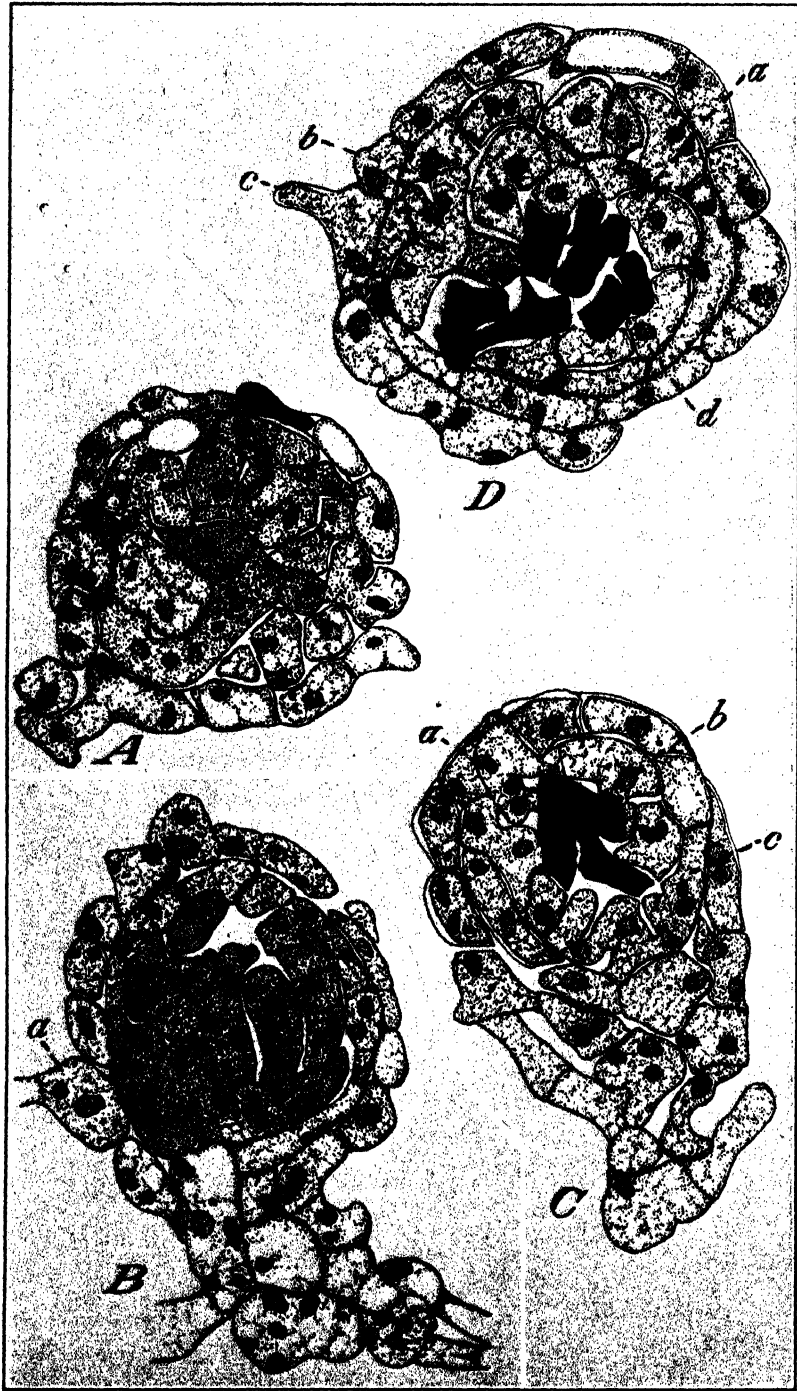
mycelium has a nucleus been found in the act of passing through a septum from one mycelial cell to another.

An attempt has been made to determine the nuclear content of perithecial cells. Such an attempt must, of course, result in an underestimate of the number of nuclei per cell, for in sectioned material many cells are cut in two, and some of their nuclei appear in one section and others in another. No attempt was made to piece the two together. For the purpose of these counts, each segment of a maze was considered a cell. The results of the counts are given in table 1.

TABLE 1.—*Number of nuclei in cells of perithecia of different ages and in different parts of perithecia*

PERITHECIA OF DIFFERENT AGES

Young perithecia (4 cells or less in diameter)			Slightly older perithecia (5 to 7 cells in diameter)		
Nuclei per cell (number)	Cells		Nuclei per cell (number)	Cells	
	Number	*Percent*		*Number*	*Percent*
1	60	60	1	131	65.5
2	33	33	2	59	29.5
3 or more	7	7	3 or more	10	5
Total	100	100	Total	[1] 200	100

DIFFERENT PARTS OF PERITHECIA [1]

Two outer layers of perithecium			Central area of perithecium		
Nuclei per cell (number)	Cells		Nuclei per cell (number)	Cells	
	Number	*Percent*		*Number*	*Percent*
1	106	69	1	25	55
2	45	29	2	14	30
3 or more	3	2	3 or more	7	15
Total	154	100	Total	46	100

[1] The same 200 cells of slightly older perithecia have been regrouped according to their location in the perithecium.

As may be seen from the data in table 1, approximately one-third of the cells in young perithecia carry more than one nucleus, and the percentage of cells with more than one nucleus in the outer layers of a perithecium is nearly as large as in the central area. Of course, if a count could be made in unsectioned perithecia, the proportion of cells with more than one nucleus would be found to be greater than one-third. If a whole maze were to be considered as one cell, nearly all (perhaps all) the cells would be counted as multinucleate.

In summing up the observations on the early stages of development of the perithecium, the conclusion seems justified that following the fusion of the two initial cells growth takes place with incomplete closure of the septa, and that through the mazes thus established nuclei (presumably both (+) and (−)) become widely distributed,

EXPLANATORY LEGEND FOR PLATE 6

A and *B*.—Older perithecia with high nuclear content and all cells living. Young appendage at *B, a*.
C.—Longitudinal section of perithecium with dead multinucleate central cell, *b*, and mazes at *a* and *c*.
D.—Transverse section of perithecium with group of dead cells at *d*, cells starting to grow centripetally at *a* and *b*, and young appendage at *c*. All × 1,400.

thus diploidizing a large part of both the outer and inner layers of the perithecium.

During the next stages of growth the oldest cell in the perithecium (i. e., the cell formed by the fusion of the first two cells at the initiation of the perithecium) dies. The time at which the death of the central cell occurs varies somewhat. In plate 6, *A* and *B*, all cells are still living, while in the younger perithecia shown in plate 5, *C* and *E*, the central cell is beginning to die. In the latter (pl. 5, *C*, *a*, and *E*, *a*) the cytoplasm of the central cell stains darkly and the nuclei are beginning to shrink. The numerous nuclei left in the central cell at this time do not leave it but die with it. In the perithecium drawn in plate 6, *C*, the central cell, *b*, is dead, but its dozen or more nuclei can still be seen there shrunken and dead.

Commonly a little later a few other cells most closely associated with the central cell also die. This is shown in plate 6, *D*, *d*.

When young perithecia with dying central cells were first encountered, it was taken for granted that they were, for some unknown reason, decadent and degenerating. When this condition was found repeatedly, such an explanation appeared more and more doubtful. It was remembered, also, that the mildew used for this study grew out of doors under conditions ideal for its development and that the mildew was making a most luxuriant growth.

In order to learn how general this death of the oldest, first-formed cells of the perithecium was, a survey was made of over 100 perithecia. The data on these were first classified according to the size of the perithecium. Perithecia five cells in diameter were placed in one group, those six cells in diameter in another, etc. Then the perithecia within each size group were classified according to whether all cells were living, only the central cell was dead, or two to six cells of the central area were dead. The results are shown in table 2.

TABLE 2.—*Classification of young perithecia according to size and to the presence of dead cells within them*

Size of perithecia (number of cells across diameter)	Perithecia with—			Total perithecia
	All cells living	Central cell dead	Two to six cells dead	
	Number	*Number*	*Number*	*Number*
5	30	8	0	38
6	12	7	0	19
7	8	8	3	19
8	6	7	7	20
9	0	4	3	7
10	2	1	7	10
11	0	0	2	2
Total	58	35	22	115

From table 2 it can be seen that in perithecia five and six cells in diameter the majority show all cells living. In about one-fourth,

EXPLANATORY LEGEND FOR PLATE 7

A.—Perithecium with shrinking dead cells in center, surrounded by layer of centripetally growing cells. × 1,400.

B.—Perithecium with centripetal layer, dead cells at *a* and *b*, and base becoming heavier. × 1,400.

C.—Diagram of older perithecium. Remnant of dead cells at *a*. Asci will soon form at *b*. × 500.

D.—Ascus showing prophase of heterotypic division. × 1,400.
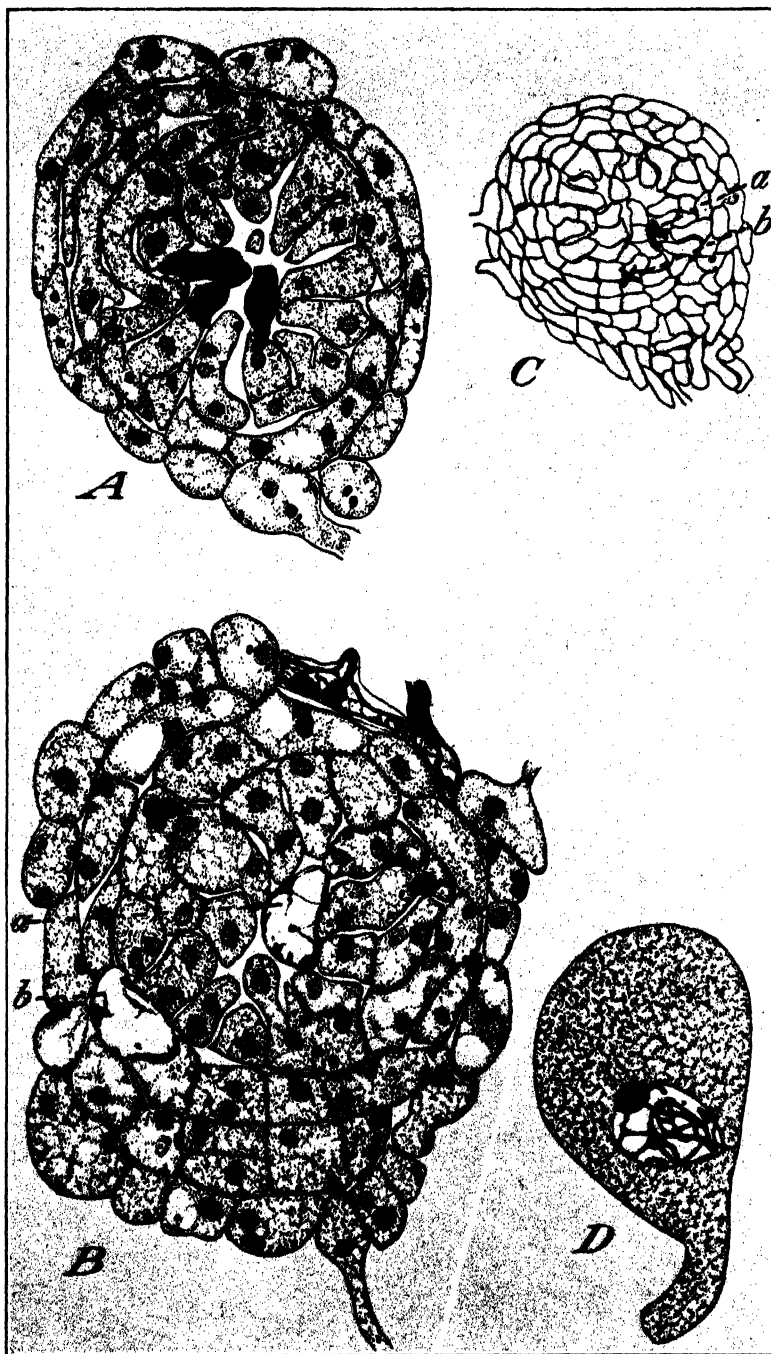
PLATE 7
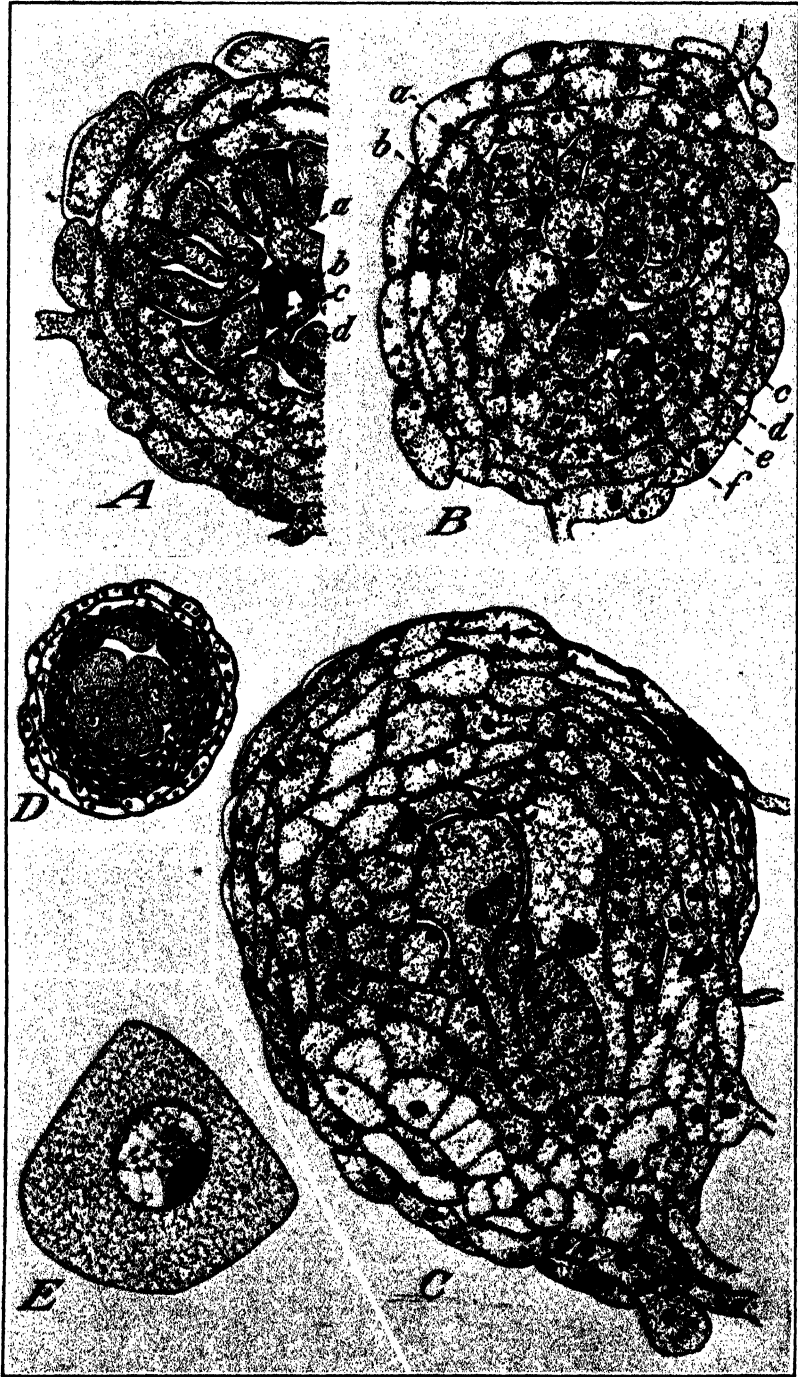
the initial fusion cell is dead. In perithecia of the seven-cell size group and larger, the number of perithecia with all cells living rapidly decreases and the number of those with more than one cell dead increases. It is exceptional to find a perithecium more than eight cells in diameter with all cells living.

This death of the oldest, first-formed cells of the perithecium does not hinder the later development. Long before these cells die many of the progeny of the two (rarely three) nuclei present in the fusion cell at the beginning have become broadly distributed throughout the growing perithecium. In the perithecium drawn in plate 6, *C*, the central cell, *b*, is dead, but the vigorous multinucleate mazes at *a* and *c* carry on the growth.

As the perithecium continues to grow, the two outer layers become differentiated as wall layers. These cells grow and divide to keep pace with the expanding perithecium. Certain of the surface cells in the equatorial region of the perithecium or slightly below it push out to form the appendages (pl. 6 *B, a*; *D, c*).

Just inside the wall of the perithecium a layer of cells grows inward, usurping the space and perhaps resorbing the substance of the dead cells. A beginning of this is seen in plate 6, *D*, where cells at *a* and *b* are turning inward. This centripetal growth is much more pronounced in the perithecium shown in plate 7, *A*, where cells all around the circle are pointed inward and the dead cells that they center upon are much shrunken.

Plate 7, *B*, shows a later stage. Although the central space is filled in solid, the layer of inward-growing cells can still be seen, while at *a* and *b* are two separated dead cells, pale and nearly empty but not collapsed. The base of the perithecium is becoming heavier as tangential divisions increase the number of cell layers.

So far as noted, in all later cell divisions in the perithecium septa are completed. No longer are openings left between daughter cells. The mazes have disappeared, suggesting that earlier openings are now closed.

Plate 7, *C*, drawn at lower magnification than *A* and *B*, shows diagrammatically the condition of the perithecium just before the formation of asci. The structure has become more massive. The central area of centripetal growth is still discernible. Between it and the surface there are now several cell layers, of which the outer layers will become the wall. Small remnants of the dead cells are seen at *a*. The asci will spring from the region indicated by *b*.

Plate 8, *A*, shows part of a section through a perithecium in which asci are beginning to form. An ascus starting at *d* forced its way upward to *a* against the pressure of the surrounding cells. It is irregular in form, being indented by each cell that it encountered in its growth. Its two nuclei at *b* are already larger than the nuclei

of nearby vegetative cells. At *c* is the shrunken remnant of one of the dead cells.

In a longitudinal section through a perithecium, two or at most three of the asci are seen. A median, transverse section cuts across all the asci. In the center of the cross section drawn in plate 8, *B*, is a nest of six asci cut at the level that shows their nuclei. The asci at *e* and *f* are the youngest and smallest of the group and are still binucleate. In the asci at *a* and *d*, nuclear fusion is in progress. In each, the two nuclei have joined but the nucleoles are still some distance apart. In the asci at *b* and *c* the nucleoles also have fused. Finer details of the nuclear fusion have not been worked out.

After the nuclear fusion the ascus enters upon a prolonged period of growth, during which it remains uninucleate. In plate 8, *C*, is shown a longitudinal section through a larger perithecium showing two uninucleate asci in the central area. In plate 8, *D*, is drawn at low magnification a transverse section through a much older perithecium. The asci have grown rapidly at the expense of the surrounding cells, which are now shrunken and dying. The lower right-hand ascus of this perithecium is drawn enlarged in plate 8, *E*. The outline is plump, the cytoplasm dense, and the large nucleus is in a prophase of the heterotypic division. In plate 7, *D*, is shown a longitudinal view of a uninucleate ascus during the later part of this growth period.

The later development within the ascus has not been studied.

DISCUSSION

The mycelium of powdery mildew is superficial, and under natural conditions is exposed to the weather. New cells formed during growth of hyphae harden quickly. When sexual reproduction begins, hyphae (supposedly (+) and (−)) are attracted to each other, as is evidenced by the formation of extensive areas of contact with each other. Rarely a nucleus moves through directly from one hypha to the other at the time and place of the first contact. It is surprising that a nucleus could effect a passage necessary for this transfer through the two older hardened walls.

The powdery mildew of sunflower (*Erysiphe cichoracearum* DC.) grows readily on separate sunflower leaves floated on sugar solution in covered Petri dishes and forms mature perithecia there with normal spores. Here the mycelium is growing indoors in diffuse light and in very humid air. At the beginning of sexual reproduction, under these protected conditions,[5] the hyphae show the same attraction for each other and form the same areas of contact as in delphinium mildew, but here the passage of a nucleus directly from one older hypha to another is fairly common. It is natural to assume that when grown in a damp chamber in this way, the cell walls of the hyphae remain thinner or softer and offer less obstruction to the passage of a nucleus than when grown out of doors. This mode of fertilization is achieved without the formation of any reproductive branches. There is nothing here that could be labeled an "antheridial" or "oogonial" branch.

[5] Unpublished data.

Occasionally the growing tips of two ordinary mycelial hyphae of delphinium mildew meet, stop growing in length, swell, curl, and an opening forms between the tips, thus initiating a perithecium. Here, also, fertilization takes place without the formation of special reproductive branches, for the joined apices of two ordinary hyphae serve as the point of communication. It may be pointed out that the apex of any hypha would have a thin, soft wall that would facilitate the formation of such an opening.

Not uncommonly the pair of cells that fuse consist of the growing tip of an ordinary mycelial hypha and a short side branch from an older part of a second hypha. One gets the impression from studying such cases that as the tip of one hypha grows past the other an attraction between the two occurs that inhibits the apex of the one from growing farther and incites the formation of one or more side branches on the other. Here, one of the two cells that fuse is a special branch formed in connection with reproduction.

Commonly, and in perhaps the majority of cases, the attraction and first contact are between mature hard-walled parts of two adjoining hyphae and each of the two pushes out a short side branch, thus bringing together young growing tips from the two.

In other words, the essential fact of sexual reproduction here is the meeting of two hyphae (perhaps $(+)$ and $(-)$), an opening between the two, and a nuclear transfer that brings into one cell two different nuclei. It would appear to depend on circumstances whether the transfer is (1) between older cells of two adjoining hyphae; (2) between the growing tips of two ordinary hyphae; (3) between one growing tip and a short side branch from an older hypha; or (4) between two short side branches, one from each of two older juxtaposed hyphae. Given attraction and contact between two hyphae, the nuclear transfer will take place, either directly, if the walls are soft enough to permit it, or after the formation of one or of two small side branches. Even when the two short side branches are formed, there is great variety in their shape, size, and relationship, and while it is usually the terminal cells of the two that fuse, it is not necessarily so. Moreover, it is not uncommon to find one of the two cells binucleate before fusion.

According to published accounts of the Erysiphaceae (*5, 7, 8, 43, 44, 46, 49*), the perithecium is regularly and uniformly initiated by the formation on adjoining hyphae of two characteristic short side branches, one bearing the antheridium, the other the oogonium. These specialized sex cells become slightly twisted on each other, an opening forms between the two at the apex, and a nucleus passes through from the antheridium to the oogonium.

Figures resembling the published drawings of antheridia and oogonia in powdery mildew are easily found in *Erysiphe polygoni*, but along with these there are so many other types as to suggest that, in this species at least, the formation of special sex cells is a matter of expediency rather than a necessity.

In a broad range of ascomycetes the occurrence of well-defined antheridia and oogonia has been recorded (*4, 5, 11, 12, 13, 20, 31, 40, 42, 44, 45, 47, 53, 57*). They differ in form in different species but are fairly uniform for a given species. The following exceptions and irregularities have been noted:

In *Lachnea stercorea* Pers., Fraser (*33*) finds that both oogonia and antheridia form but no antheridial nuclei enter the oogone. The ascogone, none the less, develops, and a normal ascocarp forms. Winge (*58*) finds the same condition in *Sphaerotheca*. Dangeard (*15, 16, 17, 18*) takes the extreme stand that, while antheridia and oogonia are of general occurrence in ascomycetes, it is in only a few of the most primitive that one or more nuclei pass from the antheridium to the oogonium. In all others no nuclear transfer occurs.

In some species (*1, 2, 3, 21, 26, 28, 29, 30, 35, 36, 60*) all individuals produce oogonia but no antheridia. Notwithstanding this, the ascogonia develop normally. Recent work shows that in some of these species every individual produces oogonia and also produces some form of conidia, oidia, microconidia, or spermatia, which replace antheridia in function. Heterothallism has been proved in several of these species. Only when (+) conidia are brought into contact with a (−) individual (or (−) conidia with a (+) individual) do fertilization and development of the ascocarp take place.

The mode of fertilization in these cases varies greatly in different species. In *Sclerotinia gladioli* Massey (*28, 29, 30*) all individuals produce microconidia and columnar receptive bodies. The microconidia cannot grow into mycelium and can function only when microconidia from a (+) individual are placed on the specialized receptive bodies of a (−) individual, or vice versa. In heterothallic strains of *Neurospora* (*21*), on the contrary, either the conidia or the microconidia of one sex can effect fertilization of an individual of the other sex, either directly or after growing into mycelium. Two microconidia, one (+) and one (−), can grow into mycelia that mate with each other and produce normal perithecia. Moreover, in recent work Dodge (*24, p. 425*) has proved that in *Neurospora tetrasperma* (normally bisexual and only facultatively heterothallic), "a bisexual mycelium can be obtained as the result of a vegetative union or anastomosing of hyphae from two unisexual mycelia of opposite sex reaction."

In *Bombardia lunata* Zickler (*60*) every individual, (+) or (−), forms both ascogones and spermogonia. A spermogonium is a single flask-shaped cell within which a dozen or more minute uninucleate spermatia are formed. This species also is heterothallic.

In *Humaria granulata* Quel. (*9*) every individual forms oogonia but no antheridia. Later work (*37, 38, 39*) shows that the species is heterothallic, for a single-spore culture remains sterile, but there are neither antheridia nor any form of conidia. Mating is achieved · by mycelial anastomoses between (+) and (−) individuals, which lead to the development of normal fruits.

In *Penicillium wortmanni* Klöcker, a homothallic species, Emmons (*32, p. 133*) finds that "a cell or slightly differentiated branch of the vegetative mycelium functions as an ascogonium, and no paired organs are to be found."

In *Humaria rutilans* Fries neither antheridia nor oogonia are formed. A knot of thickened hyphae develops and "fusions of nuclei in pairs occur in the hypothecium, constituting a process of reduced fertilization or apogamy" (*34*).

In these few examples there are all gradations from the typical sexual reproduction by means of oogonia and either antheridia or some form of microconidia to a type with complete absence of formal

sex cells. Lohwag (*51*) has brought together and discussed a wide range of material showing still further variations.

The present studies of *Erysiphe polygoni* show that even a species that regularly produces antheridia and oogonia can show other, less formal, types of reproduction, including even occasional instances of mycelial mating.

It was earlier believed that the passage of a nucleus (or of more than one when antheridia are multinucleate) from the antheridium to the oogonium was immediately followed by nuclear fusion (*8, 11, 12, 43, 44, 45, 46*).

The puzzling fact was often discussed, however, that there is also a nuclear fusion in the ascus. The existence of two nuclear fusions in one life cycle demanded an explanation. In 1907 Claussen (*13*) discovered that in *Pyronema confluens* Tul. the male nuclei entering the oogone pair with the female nuclei but do not fuse with them, that this pairing is followed by the formation of ascogenous hyphae with paired nuclei, and that these give rise to binucleate asci in which, finally, nuclear fusion takes place.

Although some writers since then still believe in the existence of nuclear fusions in both the oogone and the ascus (*31, 40, 41, 42, 47, 55, 56*), the majority believe that in the ascomycetes, as in the basidiomycetes, the initial cell fusion is not accompanied by nuclear fusion but leads into a more or less prolonged binucleate phase of development (with conjugate divisions of paired nuclei), terminated in the ascus or basidium by nuclear fusion. Swingle (*55*) has tabulated the results of nearly 60 pieces of research on this subject. In *Erysiphe polygoni* no evidence has been seen of nuclear fusion at the time of cell fusion.

In the typical ascomycete as recorded in the literature the fertilized oogone or ascogone is soon sheathed by sterile gametophytic hyphae that grow up from below. Their further growth provides the sterile portion of the fruit body. At the same time the ascogone itself gives rise to a special set of ascogenous hyphae with paired nuclei which in turn give rise to the asci.

Erysiphe polygoni differs markedly from this. Fertilization is followed by growth in which the septa remain incomplete for a considerable period, so that a large number (perhaps sometimes all) of the cells of the perithecium are in open communication with one another for a time, and many cells in all parts of the perithecium contain more than one nucleus. There is no definite limited set of ascogenous hyphae embedded in sterile growth, for much of the perithecium, including even a part of its peripheral layer, is sporophytic in character.

No reference has been found in the literature to a similar case in the ascomycetes. The nearest approach is *Neurospora tetrasperma*. Colson (*14*) finds that there are no well-defined ascogenous hyphae in the young perithecium. There is a central "fertile area" composed of multinucleate cells. Here (*14, p. 214*) "cross walls are rare and difficult to see." From this area the asci arise.

Hints of a similar condition in other ascomycetes are found in the literature. Of *Humaria granulata*, Gwynne-Vaughan says (*38, p. 142*): "The whole ascocarp, in fact, is derived from the union of (+) and (−) mycelia, and is formed of dithallic cells." Harper (*43*), Winge (*58*), and Homma (*49*) have figured cells with more than one nucleus in vegetative parts of the perithecium of different species of *Sphaerotheca*, but no significance was attached to this condition. In species of

ascomycetes with several-cell ascogones the septa between the cells may be incomplete. This was found by Harper (*44*) in *Ascobolus furfuracens* Pers., by Fraser (*35*) in *Lachnea cretea* Phil., by Schweizer (*52*) in *Ascobolus citrinus* Schw., and by Drayton (*29, 30*) in *Sclerotinia gladioli.* In these cases there was no mention of the formation of mazes in the later growth. In the beginning the ascogenous hyphae in many ascomycetes, however, are nonseptate and multinucleate. It seems probable that other ascomycetes will be found whose young fruit body is made up largely of communicating sporophytic cells derived directly from, or at least connected with, the fertilized ascogone.

In *Erysiphe polygoni* the ascogone and a few of the cells most closely associated with it usually die when the perithecium is seven or eight cells in diameter. This probably is common in ascomycetes. Harper (*44*) notes its occurrence in *Erysiphe communis* (Wallr.) Fr. and *Ascobolus furfuracens.* Blackman and Fraser (*9*) describe the same condition in *Humaria granulata.*

After the death of the ascogone and associated cells in *Erysiphe polygoni,* cells (usually multinucleate) of the inner layer of the perithecium wall grow centripetally. Centripetal growth also has been seen by Harper (*44*) in *Erysiphe* and *Ascobolus.*

Knowledge of heterothallism and homothallism in the ascomycetes is still limited. Table 3 gives a list of species (probably incomplete) that have been proved to be heterothallic.

TABLE 3.—*Species that have been proved to be heterothallic*

Species	Year	Author	Reference to literature cited
Ascobolus carbonarius Karst.	1926	Betts	(*6*)
Ascobolus magnificus Dodge	1920	Dodge	(*20*)
Ascobolus stercorarius (Bull.) Schröt	1931	Dowding	(*26*)
Bombardia lunata Zickler	1934	Zickler	(*60*)
Ceratostomella pluriannulata Hedgcock	1932	Gregor	(*56*)
Diaporthe perniciosa Marchal	1931	Cayley	(*10*)
Gelasinospora tetrasperma [1]	1933	Dowding	(*27*)
Humaria granulata Quel	1930	Gwynne-Vaughan	(*38*)
Neurospora [1] (several species)	1927 1932 1932 1934	Shear and Dodge Dodge	(*54*) (*21, 22, 23*)
Pleurage anserina (Ces.) Kuntze	1931	Dowding	(*25*)
Sclerotinia gladioli Massey	1932 1934	Drayton	(*28, 29, 30*)

[1] *Gelasinospora tetrasperma* (*27*) and *Neurospora tetrasperma* (*21*) are normally bisexual and homothallic. The ascospores are binucleate. Occasional dwarf ascospores are uninucleate and these grow into unisexual mycelium. These are heterothallic. A mating of 2 such unisexual mycelia of opposite sex is necessary for the production of perithecia.

There are other anomalous cases difficult to classify. Hüttig (*50*) found in *Glomerella lycopersici* Krüger a homothallic type which in single-spore culture produced perithecia freely, also a second type which in single-spore culture produced only small brown sclerotium-like bodies, but when mated with the first type, behaved as male. When exposed to heat, cold, or roentgen rays, still other types appeared with differing sex capabilities which "erfüllt die Forderung der Theorie der relativen Sexualität von Hartmann."

Homothallism frequently has been reported in ascomycetes. In earlier work, however, evidence was accepted as proof of homothal-

lism which since has been found to be inadequate. The absence of antheridia does not prove homothallism, for antheridia may be replaced functionally by microconidia, or even by mycelial anastomoses between individuals of opposite sex. The formation of antheridium and oogonium as branches of the same hypha does not prove homothallism, for their development may have been preceded by mycelial fusions with nuclear migrations. Even growing a single-spore culture which forms normal ascocarps does not prove homothallism unless special precautions are taken, for Dowding (*26*) has shown that mites carrying oidia can crawl between a Petri dish and its cover and so serve as agents for fertilizing cultures in closed Petri dishes. The list of reported cases of homothallism in ascomycetes would shrink considerably if judged by the present critical standards.

Evidence of heterothallism also is being scrutinized more critically. Derx (*19*) earlier reported *Penicillium luteum* (Zukal) Wehmer to be heterothallic. Recent work by Emmons (*32*) calls this in question, for "Our single spore cultures have produced abundant ascospores." Species of *Penicillium* often "become nonascosporic in culture", and Derx may have encountered a strain which in a single-spore culture failed to produce ascospores, not because it was a haploid but because of a loss of fertility in culture.

Until recently no tests for heterothallism or homothallism had been reported for the Erysiphaceae. Now, Yarwood (*59*) has proved the powdery mildew of sunflower to be heterothallic, and Homma (*48, 49*) reports that *Sphaerotheca fuliginea* (Schlecht.) Poll. is homothallic. Yarwood is now conducting the experimental work on this point in *Erysiphe polygoni*.

SUMMARY

In vegetative mycelium of *Erysiphe polygoni* DC., cells are long, straight, and uninucleate. Hyphae show no attraction for each other. There is some evidence of cytoplasmic streaming through septa.

In sexual reproduction hyphae swell toward each other, forming broad areas of contact. A nucleus may pass directly from one hypha to the other, or communication may be established at the joined apices of two hyphae or between short side branches on adjoining hyphae.

The opening between the two fusing cells broadens rapidly. No indication has been seen that nuclear fusion occurs in the fusion cell.

Cell fusion is followed by growth with incomplete closure of the septa, resulting in the formation of chains of communicating cells or mazes, which make up the bulk of all parts of the perithecium, including even its peripheral layer. These cells, evidently sporophytic in character, contain from one to several nuclei. In the later growth the septa of the mazes are completed and further cell divisions reduce the number of nuclei per cell.

When the young perithecium is seven or eight cells in diameter, the initial fusion cell and usually a few of the cells most closely associated with it die. As the perithecium expands cells of the inner wall of the perithecium grow centripetally, filling the central space. From the lower central area five or six cells grow up to form the asci. Each ascus contains at first two nuclei, which soon fuse.

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HYBRID VIGOR AND GROWTH RATES IN A MAIZE CROSS AND ITS RECIPROCAL [1]

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INTRODUCTION

With the development and rapid commercial utilization of hybrid corn, the phenomenon of hybrid vigor is becoming of increasing practical importance. The theoretical aspects of hybrid vigor have received considerable attention, but certain phases of the phenomenon have not yet been adequately explained. The two current theories as to the cause of hybrid vigor are (1) physiologic stimulation due to the interaction of unlike germ plasms and (2) dominant favorable growth factors. Observational genetic evidence and results from convergent improvement studies (*12*)[2] are in agreement with the second hypothesis.

Ashby (*1, 2*) has reported on a series of experiments which he interprets as requiring some modification of the dominant-favorable-growth-factor hypothesis and which seem not to be in accord with extensive observations on hybrid vigor in maize.

He (*1, p. 458*) suggests that hybrid vigor may be manifested in four ways:

1. More meristematic centres may be present in the hybrid, giving more leaves and tillers. 2. The leaves of the hybrid may be more efficient in photosynthesis. 3. The embryo may be bigger, so that the capital with which the hybrid germinates is bigger. 4. Finally, the falling off in the sigmoid curve of growth might occur much later for the hybrid than for its parent * * * [and concludes that] Hybrid vigour in these strains is nothing more than the maintenance of an initial advantage in embryo size.

This conclusion assumes that reciprocal hybrids differing in embryo weights must also differ in the weight of the mature plant.

Extensive observations of reciprocal maize hybrids have indicated that such hybrids are essentially similar in their general plant development unless distinct differences in seed condition are involved. Since the phenomenon of hybrid vigor in maize is of such great practical as well as theoretical importance, and since Ashby's conclusions are so contrary to the beliefs commonly held by corn breeders, it was decided to repeat Ashby's experiments in considerable detail.

MATERIAL AND METHODS

Later generations of the inbred strains of corn (*Zea mays* L.) used by Ashby (*2*), 228–4–8, 228–6–5, and their reciprocal hybrids, were employed in this study. In 1933 the material was grown at the Arlington Experiment Farm, Arlington, Va., near Washington, D. C.,

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and in 1935 at Columbia, Mo. The two seasons were very dissimilar, 1933 being quite favorable for corn and 1935 very unfavorable.

The method of planting was the same in each year, the plants being checked in hills 3.3 feet apart in each direction. The two inbred lines were compared directly, one plant of each line being grown in each hill. The same system was used for the two reciprocal hybrids. Plantings made in this manner had two advantages when inbred was compared with inbred or hybrid with hybrid: (1) Sampling errors were reduced because of the elimination of correlated variation, and (2) competition should accentuate any differences in growth rate which might exist. These advantages do not apply to the important comparison of inbred with hybrid. It is possible that this planting arrangement may actually have reduced the reliability of such comparisons.

Ten plants of each inbred and hybrid were harvested at 5-day intervals throughout the growing season. All harvests were made from perfect-stand hills, hills adjacent to a missing hill being discarded. In harvests made after silking, plant and ear weights were recorded separately. The data presented deal almost exclusively with plant weights.

A comparison of growth rates for the 1933 crop was made by two methods. The first consisted in fitting a straight line to the dry weights obtained from the first nine harvests. A measure of the significance of slope is provided by Fisher (5). The second method consisted in fitting a curved regression line of the form $Y = a + bx + cx^2 + dx^3$ . . . to the entire period of growth. The y values were the differences between the logarithms of the dry weights at each harvest period. According to Fisher's terminology, a' is the mean difference and b' and c' are proportional to the difference in slope and change in slope, respectively. The contributions of these various items (a', b', c', etc.) to the total variance can be readily measured.

EXPERIMENTAL RESULTS

1933 CROP

The dry weights of seeds, embryos, and successive plant harvests for the inbred parents and their reciprocal hybrids are available. The weights of shelled grain at successive harvests were obtained in only one season and then only for the hybrids; as these data are somewhat unrelated to the rest of the material to be presented, they will be considered first. The detailed results are presented in table 1. The yields are below normal and somewhat erratic. This may be a result of flooding; water stood 6 feet deep over these plots between August 22 and 24, at which time the ears were in the milk stage. Regardless of the variability, the grain yields of the reciprocals are not significantly different in trend or at the final harvest period.

TABLE 1.—*Dry weight of shelled grain of the hybrid 4–8×6–5 and its reciprocal at successive harvests in 1933*

Date of sampling	Weight [1] of shelled grain per plant		Date of sampling	Weight [1] of shelled grain per plant	
	4–8×6–5	6–5×4–8		4–8×6–5	6–5×4–8
	Grams	*Grams*		*Grams*	*Grams*
Aug. 7	26.1±1.27	27.5±2.97	Sept. 2	165.8±5.57	158.4± 7.12
Aug. 12	49.4±3.67	41.6±3.51	Sept. 6	149.6±7.28	146.4±11.63
Aug. 18	110.0±4.71	91.2±7.08	Sept. 11	149.4±8.81	144.4± 7.35
Aug. 29	149.1±5.60	133.8±5.39	Sept. 16	173.0±4.62	162.6± 6.12

[1] The standard error, and not the probable error, is shown.

The dry weights for seeds, embryos, and plants of the successive harvests are presented in table 2. Here, as in Ashby's results, the inbred 228-6-5 has the smaller kernel and embryo. The difference in embryo weights for the two inbreds is not significant, whereas the difference between the reciprocal hybrids is significant. The logarithms of the weights of the plants at the successive harvests are shown graphically in figures 1 and 2.

TABLE 2.—*Dry weights of seeds, embryos, and plants at successive harvests of two inbred lines of corn and their reciprocal hybrids in 1933*

Part of plant or date of harvest	Individuals in sample	Mean dry weight[1] for indicated parent or hybrid			
		4-8 selfed	6-5 selfed	4-8×6-5	6-5×4-8
	Number	*Grams*	*Grams*	*Grams*	*Grams*
Seeds	50	0.2228±0.0018	0.1891± 0.0021	0.2575±0.0012	0.2314±0.0023
Embryos	50	.0029± .0005	.0026± .0004	.0035± .0006	.0020± .0007
Plants harvested:					
May 29	10	.060 ± .012	.041 ± .004	.105 ± .002	.078 ± .005
June 3	10	.129 ± .023	.088 ± .010	.270 ± .022	.197 ± .006
June 8	10	.388 ± .042	.289 ± .010	.812 ± .059	.626 ± .031
June 13	10	1.643 ± .226	.785 ± .096	3.926 ± .206	2.511 ± .341
June 18	10	2.862 ± .430	1.369 ± .084	8.309 ± .267	6.535 ± .448
June 23	10	6.909 ± .956	2.853 ± .221	18.476 ±1.40	13.286 ±1.25
June 28	10	15.31 ± .856	10.74 ± .646	31.399 ±2.90	25.008 ± .993
July 3	10	27.51 ±2.50	18.05 ± 2.06	62.8 ±5.13	60.6 ±5.50
July 8	10	34.29 ±2.83	31.35 ± 1.36	93.0 ±5.18	86.6 ±6.28
July 13	10	44.77 ±4.61	35.32 ± 2.94	136.1 ±3.96	124.7 ±4.86
July 18	10	63.80 ±5.47	53.0 ± 3.57	167.8 ±7.70	175.2 ±4.33
July 23	10	93.25 ±5.92	63.25 ± 5.81	204.1 ±7.81	190.4 ±4.53
July 28	10	97.00 ±6.52	94.38 ±12.27	233.6 ±6.48	234.3 ±3.70
Aug. 2	10	147.25 ±5.83	115.25 ± 5.56	246.0 ±8.10	240.6 ±8.25
Aug. 7	10	175.50 ±6.78	122.75 ± 5.47	275.6 ±6.45	279.7 ±6.56

[1] The standard error, and not the probable error, is shown.

The straight-line regressions, fitted to the logarithms of the dry-weight determinations for the first 45 days for the two parental lines and their reciprocal hybrids, were as follows:

Parent or hybrid: *Value of y*
228-4-8 .... 0.07277+2.53064x
228-6-5 .... .07390+2.29566x
228-4-8 × 6-5 .... .07579+1.81698x
228-6-5 × 4-8 .... .07858+1.64024x

The differences in the slope of the four regression lines as indicated by the regression coefficients are not statistically significant. This suggests that during the 45-day period the inbreds and their reciprocal hybrids all grew at approximately the same rate.

An analysis of the data for the entire growth period by means of the curved regression line leads to essentially the same conclusion. The F values (15) for the various constants are presented in table 3. In no case is there a significant difference in slope (b'). This indicates no significant dissimilarity of growth rates during the period studied.

The difference between the mean dry weights (a') is significant in every comparison. For the reciprocal hybrids, however, the difference in mean dry weight is of limited biological significance, as the two hybrids do not differ statistically in weight after the 40-day period. In the comparisons of the change in the slope of the regression lines (c'), the inbred lines in each case differ significantly from the hybrids because of the earlier cessation of growth in the hybrid (fig. 1). With the exception of the failure to find a continuing difference between reciprocal hybrids, the results are in apparent harmony with those presented by Ashby.
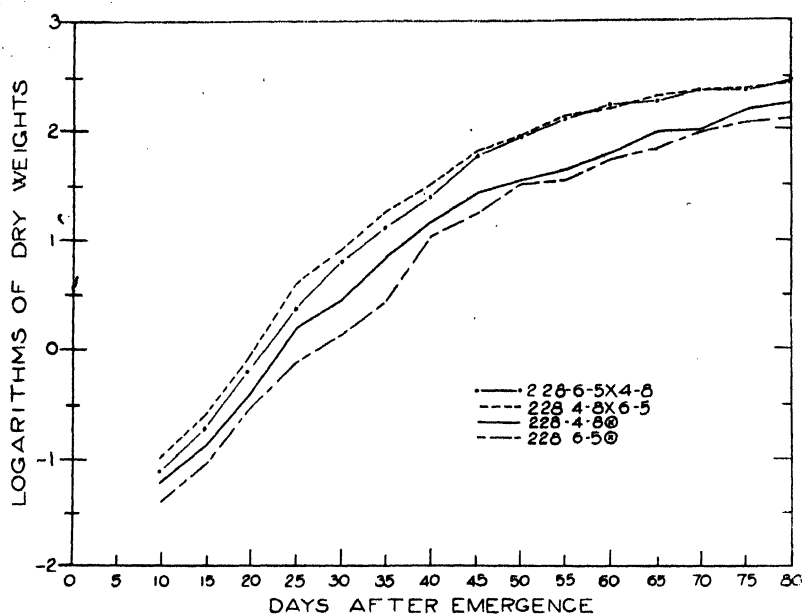
FIGURE 1.—Growth rates of two inbred strains of corn and their reciprocal hybrids in 1933.
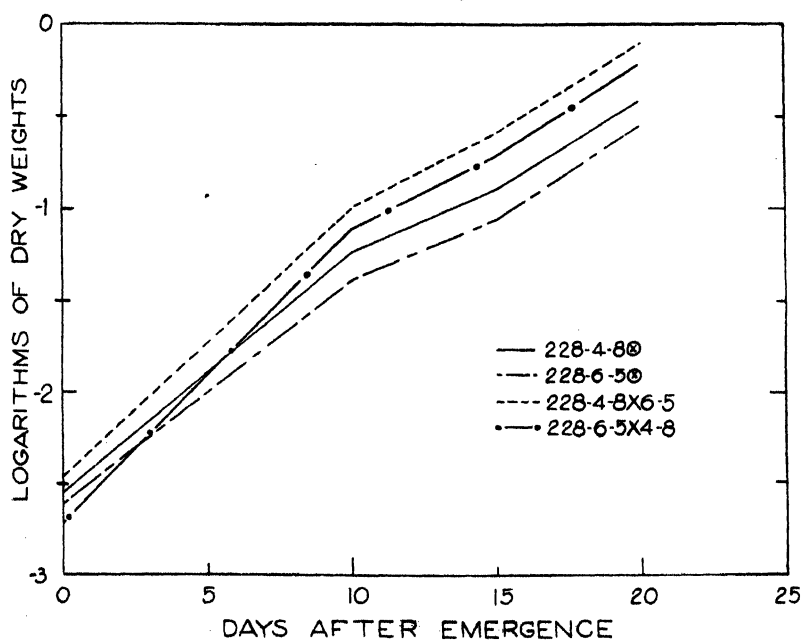
FIGURE 2.—Growth rates of two inbred strains of corn and their reciprocal hybrids during the early seedling stage in 1933.

TABLE 3.—*Comparisons of the regression constants of two inbred lines of corn and their reciprocal hybrids grown in 1933*

Comparison	Value [1] of *F* for indicated constant		
	a'	*b'*	*c'*
4–8 selfed and 6–5 selfed_____	50. 68	0. 40	0. 43
4–8 selfed and (4–8 × 6–5)_____	562. 16	. 04	19. 94
6–5 selfed and (6–5 × 4–8)_____	274. 35	. 00	11. 35
(4–8 × 6–5) and (6–5 × 4–8)____	18. 18	1. 00	. 22

[1] 4.84 is the lowest value *F* may take for significance when *P*=0.05; 9.65 is the lowest value *F* may take for significance when *P*=0.01.

Before proceeding further with the analysis it is desirable to evaluate the reasonableness of the indicated conclusions. If the dry weight at the end of the 45-day period is expressed as a percentage of the initial weight, the values for the inbreds 4–8 and 6–5 and the hybrids 4–8 × 6–5 and 6–5 × 4–8 are 572, 765, 886, and 1,110, respectively. When each hybrid is compared with its maternal inbred, the ratio of these percentages is approximately 1.5 to 1. This indicates that the hybrid actually has grown at a rate approximately 50 percent greater than its maternal inbred parent. The failure to establish the significance of this difference may be due to several causes. The sampling may have been inadequate to represent the population, or the variability from sample to sample so great that a very large difference in growth rate would be necessary for significance.

On the basis of the variability observed in the nine harvests covering the initial 45-day period, a difference in growth rate of 75 percent would be necessary to give odds of 20 to 1. As indicated previously, a difference of approximately 50 percent was observed. The difference necessary for significance covering the entire growth period has not been calculated but it doubtless would be somewhat greater than for the 45-day period.

The results published by Ashby appear less conclusive when one considers the percentage increase in dry matter as a measure of growth rate. Expressing the final harvest weight as a percentage of the initial harvest weight and comparing the faster growing inbred with its F_1 hybrid, differences of 21 and 46.9 percent are found for the material presented in his first and second study, respectively. In each case he concluded that the inbred and hybrid were equivalent in growth rate. It is clearly evident that the high degree of variability present in Ashby's and in the writer's material precludes any very definite conclusions as to the equivalence of growth rate in inbreds and hybrids.

_{1935 CROP}

The detailed data for the 1935 harvests are presented in table 4 and are shown graphically in figure 3. In contrast to the 1933 data, the inbred 228–6–5 has the higher embryo and grain weight. The hybrid 6–5 × 4–8 has a smaller grain and embryo weight than the maternal inbred itself. The reciprocal hybrid, however, shows an increase in both grain and embryo weight over its maternal inbred parent. These variations in embryo weight are probably the result of variation in soil fertility and relative plant development. Selfed

seed produced on a favored inbred plant may have larger kernels and embryos than hybrid seed produced on a less favored plant of the same inbred strain.
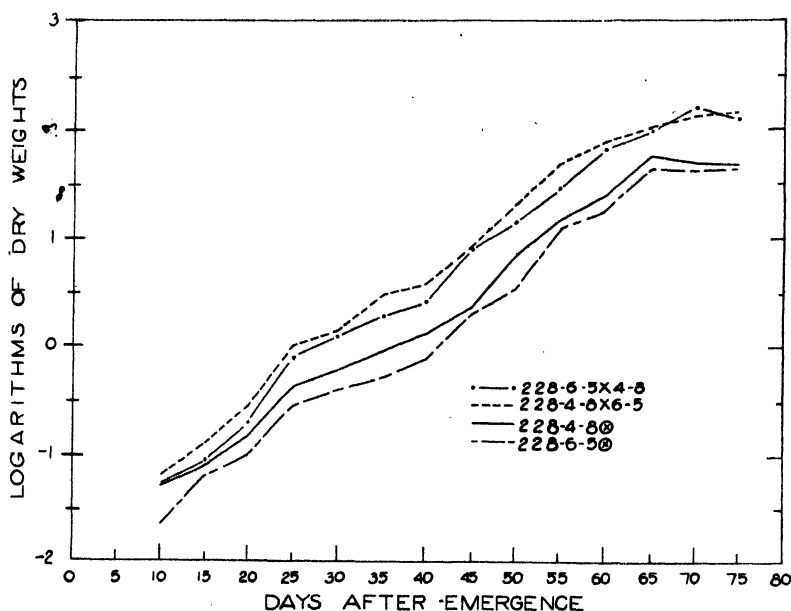
FIGURE 3.—Growth rates of two inbred strains of corn and their reciprocal hybrids in 1935.

TABLE 4.—*Dry weights of seeds, embryos, and plants at successive harvests of two inbred lines of corn and their reciprocal hybrids in 1935*

Part of plant or date of harvest	Mean dry weight [1] for indicated parent or hybrid			
	4–8 selfed	6–5 selfed	4–8 × 6–5	6–5 × 4–8
	Grams	*Grams*	*Grams*	*Grams*
Seeds	0.215 ±0.007	0.265 ±0.001	0.246 ±0.002	0.225 ±0.001
Embryos	.0031± .0003	.0034± .0005	.0035± .0003	.0029± .0002
Plants harvested:				
June 18	.053 ± .003	.024 ± .001	.066 ± .003	.054 ± .002
June 23	.096 ± .007	.060 ± .004	.136 ± .005	.086 ± .003
June 28	.159 ± .039	.104 ± .011	.284 ± .020	.201 ± .013
July 3	.434 ± .015	.302 ± .006	1.027 ± .030	.825 ± .079
July 8	.578 ± .025	.427 ± .012	1.401 ± .110	1.317 ± .034
July 13	.902 ± .074	.538 ± .009	3.191 ± .248	2.086 ± .191
July 18	1.307 ± .116	.719 ± .039	3.786 ± .217	2.612 ± .189
July 23	2.433 ± .287	2.179 ± .294	8.444 ± .562	8.111 ± .959
July 28	7.110 ± .413	3.571 ± .448	21.700 ±1.277	14.500 ± .633
Aug. 2	15.69 ± .563	12.679 ± .464	47.662 ±3.543	37.297 ±2.602
Aug. 7	25.116 ±1.902	18.900 ±1.489	77.116 ±1.704	72.137 ±5.304
Aug. 12	58.548 ±1.277	44.506 ±2.075	105.971 ±2.546	99.930 ±3.405
Aug. 17	48.848 ±1.410	45.415 ±1.033	132.619 ±2.049	153.614 ±8.702
Aug. 22	50.482 ±4.549	45.512 ±4.593	141.288 ±6.345	128.460 ±8.434

[1] The standard error, and not the probable error, is shown.

The difference in weight of embryo for the hybrid 4–8 × 6–5 and its reciprocal was not very great in either year. In order to obtain a greater difference in embryo weight, one set of the hybrid ears 6–5 × 4–8 was harvested April 20 and another set was harvested

May 4. This material was grown in the greenhouse and the sequence of silk and pollen prevented making the cross reciprocally. The difference between the embryo weights of the prematurely and normally harvested corn was slightly greater than that of the two lots of reciprocal hybrids previously compared. This initial difference in embryo weight, however, was ineffective in modifying the expression of vigor. The mean dry weights of plants (table 5) from the two lots of seed are quite similar for practically every harvest. There is no significant difference between the final dry weights.

TABLE 5.—*Mean dry weights of seeds, embryos, and plants at successive harvests of the hybrid (6-5 × 4-8) harvested before and at maturity in 1935*

Part of plant or date of harvest	Mean dry weight [1] for the hybrid 6-5 × 4-8 at—		Part of plant or date of harvest	Mean dry weight [1] for the hybrid 6-5 × 4-8 at—	
	Premature harvest [2]	Normal harvest		Premature harvest [2]	Normal harvest
	Grams	*Grams*		*Grams*	*Grams*
Seeds	0.214 ±0.001	0.278 ±0.002	Plants har-vested—Contd.		
Embryos	.0027± .002	.0036± .0002	July 23	13.111±0.926	14.777±1.445
Plants harvested:			July 28	33.300±1.352	43.300±2.553
June 18	.054 ± .002	.065 ± .003	Aug. 2	76.319±3.600	80.734±1.910
June 23	.112 ± .006	.124 ± .008	Aug. 7	107.064±1.524	113.034±4.068
June 28	.288 ± .017	.342 ± .022	Aug. 12	130.846±2.170	167.182±6.135
July 3	.933 ± .056	.883 ± .035	Aug. 17	145.350±3.116	180.586±7.259
July 8	1.719 ± .059	1.891 ± .058	Aug. 22	169.565±6.384	163.583±6.426
July 13	3.757 ± .277	3.848 ± .274			
July 18	5.138 ± .468	5.353 ± .807			

[1] The standard error, and not the probable error, is shown.
[2] The premature harvest was 2 weeks earlier than the normal harvest.

The evaluation of the curved regression constants is presented in table 6. The difference between mean dry weights (a') is significant in every comparison. However, as pointed out for the 1933 results, the difference between the reciprocal hybrids is of little practical importance. The final plant weights were not significantly different. The difference in rate of growth (b') for the two inbreds probably is significant, the value for F lying between 0.01 and 0.05. The difference in growth rate between the 228–4–8 inbred and the hybrid 4–8 × 6–5 is clearly significant. The inbred 228–6–5 and the hybrid 6–5 × 4–8 do not differ significantly in slope, though the F value approaches the 5-percent point. The ratio of the percentage growth for this hybrid and its maternal inbred for the initial 45-day period is 1.8 to 1.0, or an indicated difference in growth rate of 80 percent.

TABLE 6.—*Comparisons of the regression constants of two inbred lines of corn and their reciprocal hybrids grown in 1935*

Comparison	Value of F_1 for indicated constant		
	a'	b'	c'
4–8 selfed and 6–5 selfed	41.272	5.491	0.183
4–8 selfed and (4–8 × 6–5)	387.172	15.953	21.018
6–5 selfed and (6–5 × 4–8)	279.818	4.249	6.375
(4–8 × 6–5) and (6–5 × 4–8)	19.324	3.901	1.505
(6–5 × 4–8) from prematurely and (6–5 × 4–8) normally harvested seed	16.211	.028	.144

[1] 4.96 lowest value F may take for significance when $P=0.05$. 10.04 lowest value F may take for significance when $P=0.01$.

DISCUSSION

In a recent study Engledow and Pal (*4*) concluded that Ashby's explanation of hybrid vigor in maize was inapplicable to wheat. The following discussion is concerned with the agreement and discrepancies of the data presented here and the expectations based on Ashby's conclusions. The discussion will be facilitated by considering each of his postulates separately. His first postulate is that the F_1 hybrid of a cross between two inbreds exhibits the growth rate of the faster growing parent. In a consideration of this postulate it is desirable to make some subdivision of the growth period. The following subdivision is in harmony with the sampling periods and appears useful: (1) From fertilization to maturation of the seed, (2) from germination through the early seedling stage, and (3) from the late seedling stage to mature plant. The first two stages are essentially similar and are subdivided only because they are separated in time by the dormancy of the mature embryo. It should be emphasized that Ashby's studies were concerned almost entirely with the third stage of growth.

Inbred and hybrid embryos can be produced on the same ear and must therefore have their origin in egg cells of the same average weight. As any conceivable difference in weight of sperms from the two types of pollen could not cause more than a trivial difference in weight of the zygote, the inbred and hybrid zygotes are necessarily of similar weight. Since hybrid embryos in general are heavier than selfed embryos,[3] it is clear that the logarithmic growth lines must differ in their slope in the first period. A large amount of data relating to the mature weights of selfed and hybrid seeds has been presented by Kiesselbach (*8*).

Information regarding the second phase of growth is very meager. However, on the basis of the data illustrated in figure 2, it appears that during germination and for at least the first 15 days of seedling growth the rates for inbred and hybrid are decidedly different.

The extensive data dealing with the third stage of growth cannot be used as a critical test of this postulate. Under conditions in which sampling errors obscure differences in growth rates of the magnitude of 50 percent any conclusions as to the equivalence of growth rate in inbred and hybrid are unwarranted.

Lindstrom (*10*) has presented data which he interprets as a disproof of Ashby's hypothesis. He removed a portion of the leaf area of the hybrids at different stages and compared their final growth with that of the inbred parents. The F_1 hybrids exceeded the inbred parents in final plant weight, and he concludes that "This can only mean that the cut-back F_1 hybrids grew at a faster rate than their untouched parents." The writer is in agreement with the idea that F_1 hybrids grow at a faster rate than their parental inbreds. There is some doubt, however, that this has been conclusively proved by the above experiments. The clipping of leaves does not remove "capital" in the sense in which Ashby and physiologists in general have used the word. The tissues removed are no longer meristmatic but are fully differentiated and have attained approximately their ultimate size. If one considers the clipping to have affected only the "net rate of interest" or the "efficiency index" then the results are not necessarily at variance with Ashby's conclusions. The reduction in leaf area and conse-

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[3] This statement applies only to inbred or closely line-bred material.

quently the reduction in efficiency index would be expected to be temporary. Even under conditions in which the hybrid and inbred grow at exactly the same rate, the hybrid starting with the larger capital could suffer a temporary reduction in efficiency index and still produce a greater final weight than the inbred parents. But, assuming that the tissues removed by clipping of leaves do represent capital, data showing that the dry matter of the decapitated hybrid was reduced to equivalence with the parental inbred would be essential before one could consider a difference in growth rate as having been proved.

Although the present data are in many respects inadequate, they do indicate that for the first two phases of growth the rate for the hybrid is in excess of that for either parent. It has not been possible to demonstrate a statistically significant difference in growth rate of hybrid and inbred during the third period, but it has been demonstrated that only very large differences would appear significant because of the large sampling errors involved. The data suggest that the difference in growth rates between inbred and hybrid may not be as great during this stage as in the two earlier stages. A possible explanation for this may be in the type of growth involved. Within 25 to 35 days after planting, the morphological foundation of the corn plant is largely completed; the growing point becomes differentiated into a tassel, and growth by cell division becomes of minor importance. Growth by cell differentiation and enlargement has been increasing in importance during the period and from this point on becomes the dominating element.

The presence of two distinct phases of growth in the life of an organism appears to be a common phenomenon in biology. Huxley (*7, p. 118*) in this connection states:

In the first, the general form of the part is being laid down, and this process is accompanied by very rapid alterations of form, and by marked histological changes; in the second, histological changes are absent or of an entirely secondary nature, and the form changes are confined to quantitative alterations in the proportion of the definitive structural plan. * * * Thus not only definitive form-plans, but also marked differences in size, are established in the short first phase, and effects of growth during the second phase are confined to a quantitative modification of the already diversified organization given at the close of the first phase.

Similar growth rates during a particular stage of development have been reported for markedly differing size structures in a wide variety of organisms. The works of Houghtaling (*6*) on tomatoes, of Sinnott and Kaiser (*14*) on *Cucurbita*, and of Dobzhansky and Duncan (*3*) on *Drosophila* larvae are only a few of the many cases that might be cited.

Ashby's second postulate, namely, that rate of growth is inherited as a simple dominant or dominant complex contributed by one of the parents, does not appear to be true for either the first or second growth period. Because of the large sampling errors, the data presented here on the third growth period cannot be considered as critical. However, every person who has had experience with corn breeding can cite numerous instances of the expression in the hybrid of characters from the two parents that certainly are related to growth rate, as, for example, leafiness and the possession of a good root system.

Ashby's third postulate, namely, that reciprocal hybrids differing in embryo weight should differ in degree of vigor, is only partially

substantiated. The initial difference between such hybrids persists for a time but disappears before the completion of growth. The results for both seasons are in agreement on this point. It seemed desirable to compare the growth rate of hybrids in which greater differences in embryo weights were involved. These differences were obtained by comparing normal and prematurely harvested seed. The data from this source are presented in tables 5 and 6. Here again initial differences in embryo size failed to persist. It has not been possible to show a difference in growth rate in such cases, but it seems probable that the explanation for the failure of such differences to persist lies in the slightly longer growth period of the hybrid having the smaller embryo. In every case such a hybrid comes into sexual maturity slightly later than its reciprocal having the larger embryo. These results are in essential agreement with those presented by Passmore (*11*) on reciprocal *Cucurbita* hybrids.

In the present experiments, variations in embryo size within a genotype have not been of importance. This is in agreement with the work of Kotowski (*9*), on peas, beans, and cabbage; Passmore (*11*), on *Cucurbita*; and Robinson (*13*) and others, on corn.

Ashby's fourth postulate, namely, that hybrid vigor is nothing more than the maintenance of an initial difference in embryo size, is not supported by these results. In both 1933 and 1935 the hybrid 6–5 × 4–8 actually had a smaller embryo than the inbred 228–6–5. As this hybrid and its maternal inbred have been shown to have the same efficiency index within the limits of sampling error, this postulate would require that the hybrid be inferior to or at least no better than the inbred. Actually the hybrid weighed more than twice as much as the inbred parent at maturity. The increased size of the hybrid is in part accounted for by a more rapid growth rate during the second growth phase.

Ashby was of the opinion that his results required some modification of the dominant-favorable-growth-factor hypothesis. This does not appear to be essential. His results could be interpreted quite simply on the assumption that the vigor of the hybrid is due to the inheritance of a large embryo size from one parent and a rapid growth rate from the other. Each of these components is the resultant of the action of a large number of factors. The dominant-favorable-growth-factor hypothesis does not require that the hybrid exceed the parents in every respect, but rather that the combined effect of all factors shall produce a greater end product. The most favorable factors contributed by each parent find expression in the hybrid. This interpretation is in accord with the fact that favorable genic modifications tend to be dominant.

The results of these experiments are of some interest in connection with the two theories on the nature of hybrid vigor. The differing growth rates during the various growth stages find a ready explanation on the basis of the dominant-favorable-gene hypothesis, in the assumption that genes have a specific time of action. The more rapid growth rate of the hybrids during the first two stages of growth would thus be attributed to the complementary action of genes which produce their characteristic effect early in the development of the organism.

The results are more difficult to explain on the theory that hybrid vigor is due to a physiologic stimulation resulting from unlike germ

plasms. If the data presented can be considered as typical, then one must assume that stimulation increases from the first to the second growth period and decreases with the third.

The mechanism by which hybrid vigor is expressed is still unknown, but, as the difference in growth rate between hybrid and inbred is greatest during the period when size increase is primarily by cell division, it is suggested that rate of cell division must be one important factor. Further studies on this problem are under way.

SUMMARY

Data are presented on the growth rates of two inbred strains of corn and their reciprocal hybrids.

The growth period from fertilization to maturity of the corn plant is divided into three phases: (1) From fertilization to maturity of seed, (2) from germination through the early seedling stage, and (3) from the late seedling stage to maturity.

By means of this classification it is shown that the hybrids grew faster than either parent during the first two phases of growth, but differences in the rate of growth during the third period have not been established statistically because of large sampling errors. The differences in the amount of growth are quite significant.

In the first two phases, the inheritance of growth rate cannot be ascribed to a complex from one parent only. The rate must represent the action, complementary or additive, of factors from both parents. The data presented are not critical for the third period.

The reciprocal hybrids were alike in growth rates and in total amounts of growth. Within a genotype, embryo weights were found to be of little importance in determining final weight, as initial differences in embryo weight failed to persist.

Hybrid vigor cannot be attributed to the maintenance of an initial difference in embryo size.

The differing growth rates for the three phases of growth are readily explained on the basis of the complementary action of dominant genes.

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THE OPTICAL ROTATORY POWER OF EXTRACTS OF DERRIS AND CUBE ROOTS[1]

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INTRODUCTION

Several years ago the author (8)[2] stated that the rotenone content of derris and cube roots could not be determined by measuring the optical rotatory power of their extracts. Other components of unknown optical activity are present, and hence the values obtained differ widely from the actual rotenone content determined by extraction. The possibility remained, however, that such a determination might give an indication of the insecticidal value of these materials. The appearance of recent articles on this subject has stimulated interest in this possibility. Danckwortt, Budde, and Baumgarten (3), for example, have proposed a method of determining toxicity of derris roots that is based on the optical rotatory power of their benzene extracts. Rowaan (12) criticized this method, stating that dextrorotatory materials are present and interfere with the results. Danckwortt (2) replied that values obtained by optical rotation correspond with the effectiveness of the root. Fischer and Nitsche (5) have recently stated that a polarimetric method gave the best indication of the insecticidal effect of a number of derris and rotenone preparations against certain insects.

Some time ago the author, in cooperation with other members of the Bureau of Entomology and Plant Quarantine (9), reported a study of the relations between certain chemical determinations and the insecticidal effectiveness against houseflies of a number of samples of derris and cube roots. Determinations were made of the optical rotatory power of acetone and benzene extracts of these samples, but the results were not included with the other data. In view of the present interest in this subject, it now seems appropriate to publish this information, together with more recent data on the optical rotatory power of extracts of several additional samples.

FIRST SET OF SAMPLES

METHODS AND RESULTS FOR ACETONE AND BENZENE EXTRACTS

The determination of the toxicity to houseflies of the first set of samples and the computation of a value for "rotenone" based on toxicity have already been described (9). This value represents the effectiveness of the root in terms of the toxicity of rotenone to houseflies.

Determinations were made of the optical rotatory power of acetone and benzene extracts of derris and cube roots that had been so prepared that 100 cc was equivalent to 10 g of the original plant material. A saccharimeter and either 1- or 2-dm tubes were used

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[1] Received for publication July 3, 1936; issued January 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 838.

(831)

for this purpose. Acetone extracts were usually slightly cloudy and often required the use of 1-dm tubes. Benzene extracts of cube root were found to be more highly colored than those of derris, and hence necessitated the use of 1-dm tubes in several cases. The concentration of rotenone equivalent to the rotation was calculated, as described in a previous article (*10*), from the following formula:

$$\text{Concentration (grams per 100 cc of extract)} = \frac{\text{Ventzke reading} \times 0.3466 \times 100}{\text{Length of tube (dm)} \times [\alpha]_D^{20}}$$

For rotenone in acetone $[\alpha]_D^{20} = -105°$. In benzene solution the optical activity of rotenone changes markedly with change in concentration, being represented by the expression $[\alpha]_D^{20} = (1.8 \times \text{concentration} - 233)°$. In the case of the benzene extracts this expression was substituted for $[\alpha]_D^{20}$ in the formula. Since the extracts contained the equivalent of 10 g of plant material per 100 cc, the values obtained were multiplied by 10 to obtain the percentage of "rotenone" in the roots equivalent to the optical rotatory power. The values for optical rotatory power and for equivalent "rotenone", together with the "rotenone" based on toxicity, rotenone by extraction, and total acetone and benzene extracts, are given in table 1.

TABLE 1.—*Comparison between optical rotatory power and other determinations of derris and cube roots. First set of samples*

Sample no.	Material	"Rotenone" based on toxicity	Rotenone by crystallization from CCl₄	Rotenone plus dequelin by Gross-Smith test	Acetone extraction			Benzene extraction			"Rotenone" from optical rotatory power of acetone and benzene extracts	"Toxic value" from rotenone and total benzene extractives
					Total extractives	Optical rotatory power[1][2]	"Rotenone" from optical rotatory power	Total extractives	Optical rotatory power[1]	"Rotenone" from optical rotatory power		
		Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Degrees Ventzke*	*Per-cent*	*Per-cent*	*Degrees Ventzke*	*Per-cent*	*Per-cent*	*Per-cent*
401	Derris root	5.5	0.0	0.8	12.2	+1.4	(3)	9.4	-3.7	2.8	7.0	4.7
523	...do.[4]	6	1.1	3.0	12.0	-1.8	3.0	10.4	-6.2	4.6	6.0	5.8
524	...do.[5]	11.5	2.0	3.2	22.4	-1.8	3.0	20.0	-10.6	7.9	12.1	11.0
594-B	...do.	11.5	5.0	14.4	21.2	-5.6	9.2	17.7	-12.7	9.5	9.8	11.4
2217-M-1	...do.	15	7.8	12.0	24.8	-6.4	10.6	21.2	-15.9	11.9	13.1	14.5
2217-P-1	...do.	9.5	3.6	5.4	22.4	-1.6	2.6	18.5	-10.9	8.2	12.8	11.0
584	Cube root	10.5	4.1	11.0	18.2	-7.2	11.9	17.3	2 -14.0	10.5	9.3	9.4
674	...do.	6	.8	3.0	14.1	-4.6	7.6	13.0	2 -8.4	6.3	5.2	5.7
686-A	...do.	18	12.1	18.6	25.4	-11.8	19.5	24.8	-22.4	16.8	14.7	17.2
2218-H-1	...do.	11	5.6	10.8	21.7	-8.0	13.2	20.3	-16.0	12.0	11.0	11.5
2218-M-1	...do.	8.5	3.8	9.6	23.0	-5.6	9.2	20.9	2 -12.2	9.1	9.0	10.6

[1] 100 cc ⊃ 10 g of plant material; calculated for 2-dm tube.
[2] Determinations made in 1-dm tube.
[3] Not calculable.
[4] *Derris elliptica.*
[5] *Derris malaccensis.* In other cases species was not identified.

In figures 1 and 2 the values for "rotenone" equivalent to the optical rotatory powers of acetone and benzene extracts, respectively, have been plotted against the values for "rotenone" based on toxicity. In about half the samples the acetone-extract values differed widely from the toxicity values. The benzene extracts gave values much closer to the insecticidal values, but they were in general lower.

COMBINED VALUE BASED ON RESULTS FOR BOTH ACETONE AND BENZENE EXTRACTS

Since the optical activity of rotenone in acetone is quite different from that in benzene, it was thought of interest to calculate the theoretical "rotenone" content from the optical activity of both acetone and benzene extracts and compare such values with the toxicity to houseflies. The optical rotatory power of both acetone and benzene extracts might be considered to be made up of that of rotenone and rotenonelike compounds plus that of dissimilar optically active material. For the purposes of this calculation it might be supposed that the optical rotatory power of all the rotenonelike compounds present is greater in benzene than in acetone to the same degree as is that of rotenone, and that the rotation of the other optically active
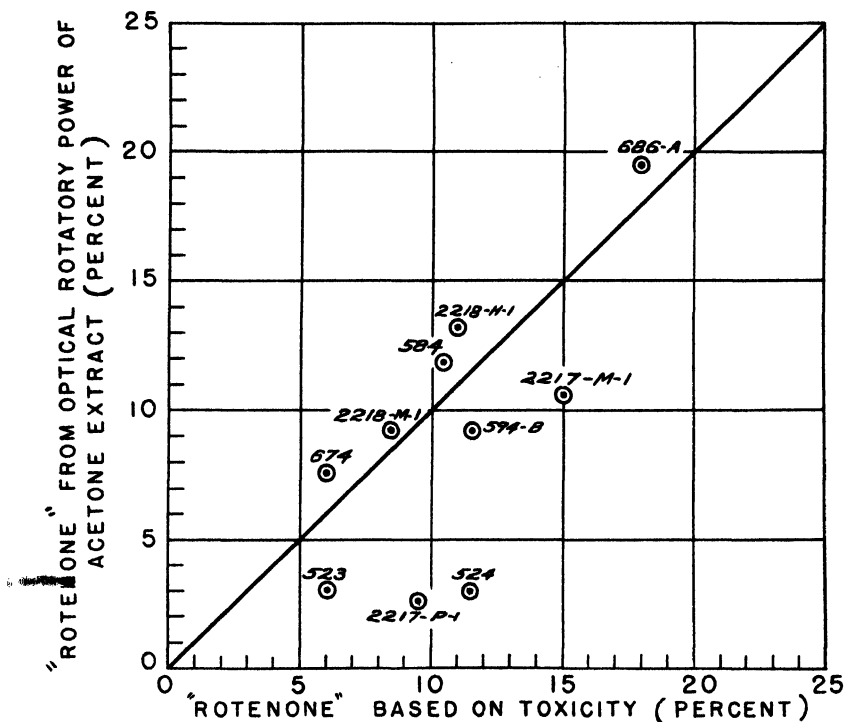
FIGURE 1.—"Rotenone" from optical rotatory power of acetone extracts versus "rotenone" based on toxicity to houseflies.

material is the same in both solvents. For this purpose it must be assumed that acetone and benzene extract the same optically active material from a given sample of root.

The following formula would give such a value for theoretical "rotenone" content:

$$\text{Concentration (grams per } 100 \text{ cc extract)} = \frac{35.51 - \sqrt{1,261 - 9.615}}{(OR_a - OR_b)}$$

where OR_a and OR_b are the observed rotations of acetone and benzene extracts, respectively, in Ventzke degrees in 2-dm tubes. The values

obtained, converted to percentages, are given in table 1. Approximate values could have been obtained by assuming the optical rotatory power of the rotenonelike compounds in benzene to be exactly double that in acetone.

In figure 3 the values calculated from this formula have been plotted against the values for "rotenone" based on toxicity to houseflies. The two sets of values are in better agreement than when calculated separately from the rotatory powers of acetone and benzene extracts. Since little is known of the optical rotatory power of the components of the extract other than rotenone, the calculation is open to question, and the only justification for discussing it is the fair agreement with toxicity values. The agreement with insecticidal value is not so good,
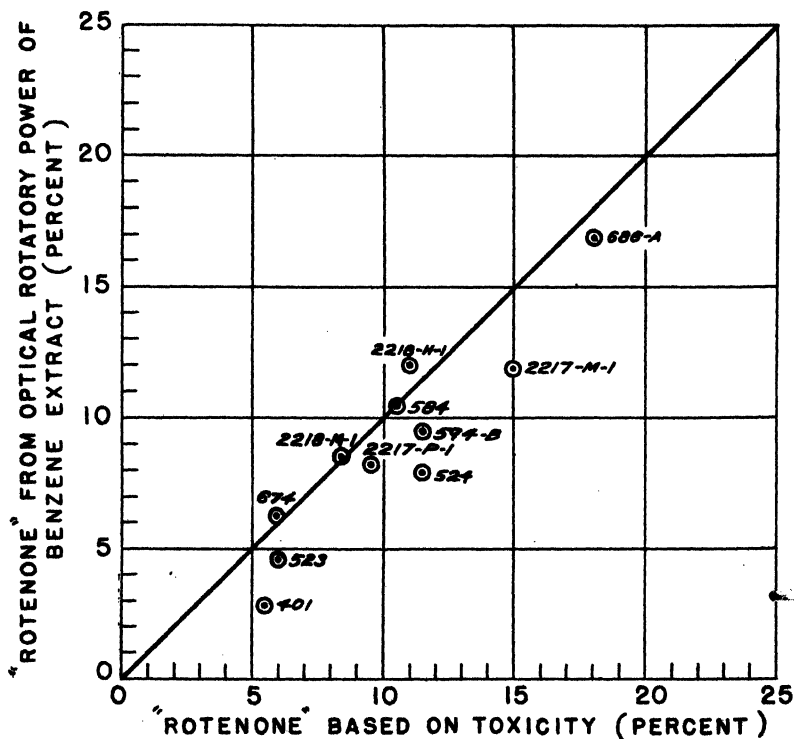
FIGURE 2.—"Rotenone" from optical rotatory power of benzene extracts versus "rotenone" based on toxicity to houseflies.

however, as in the best method referred to in the work already cited (*9, fig. 38, F*).

It might be supposed that the method would be of value as an approximate determination of toxicity, but the calculation recently proposed by Jones and Smith (*11*), based on rotenone and total extractive contents, gives an approximate value more simply and logically than the use of optical activity, which is so largely dependent on unknown constituents. Results for toxic value by this method of calculation, based on rotenone and total benzene extractives, are given in the last column of table 1 for comparison with the values obtained from optical rotation.

DISCUSSION

In table 1 it will be noted that there is no agreement between the rotenone by crystallization and the value obtained from the optical rotatory power of either acetone or benzene extract. Likewise there appears to be no constant relation between the quantity of total extractives and the optical rotation values. In two derris samples and all except one of the cube samples the values obtained from optical rotatory power by all three methods of calculation were approximately in agreement. Since the acetone extract of the derris root contáining
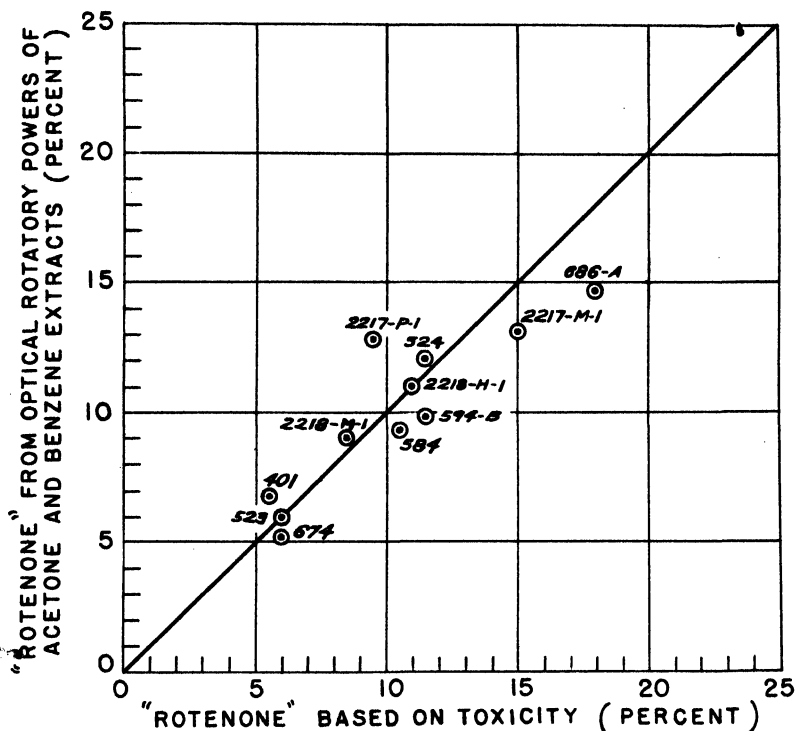
FIGURE 3.—"Rotenone" from combined optical rotatory powers of acetone and benzene extracts versus "rotenone" based on toxicity to houseflies.

no rotenone (sample no. 401) was dextrorotatory, no values for "rotenone" could be calculated.

Danckwortt and his coworkers (*3*) have stated that, since optically active deguelin possesses (accórding to Takei) about 10 percent as much optical activity in benzene as does rotenone, and since in certain tests to which they refer it is about one-tenth as toxic as rotenone (according to Takei and Gersdorff), the optical rotatory power of the benzene extract should give a good indication of toxicity. This assumes that rotenone and deguelin account for practically all the optical rotatory power, and it is stated by Danckwortt that when 10 percent of the deguelin content is added to the rotenone content the results should agree with the values calculated from benzene rotation. It has already been shown by Haller and LaForge (*7*) that Takei's material was not active deguelin but apparently only a mixture of

inactive deguelin and rotenone. Hence it is not surprising that when the calculation suggested by Danckwortt is applied to the rotenone and the rotenone-plus-deguelin values by the Gross and Smith test in the first series of roots (table 1), the results are widely different from the values obtained from the optical activity of the benzene extracts, as well as the other two optical rotation methods. Furthermore, the toxicity tests to which Danckwortt refers were made on fish rather than insects, and Gersdorff's work was done with optically inactive deguelin which, according to the more recent work of Gersdorff (6), and that of Fink and Haller (4), might be expected to be less toxic than optically active deguelin.

SECOND SET OF SAMPLES

METHODS AND RESULTS

From the chemical standpoint, for comparison with the results obtained on the first set of samples, it was thought advisable to determine the optical rotatory power of extracts of a number of additional samples of derris and cube roots. For this purpose five samples of derris root containing rotenone, five samples of cube root, and five samples of derris root containing no rotenone were selected. It should be mentioned here that the last five roots, and also no. 401 and no. 524 of the first set, were tested for "hidden" rotenone by the method of Cahn and Boam (1). The results of this test and of later determinations by the author's improved crystallization procedure indicate that some of the "no rotenone" samples may contain very small quantities of rotenone, in no case exceeding 0.5 percent. For the purpose of this work, however, the rotenone content of these samples may be considered to be negligible. The optical rotatory powers of acetone and benzene extracts were determined by the method outlined for the first set of roots, and similar calculations were made.

TABLE 2.—*Comparison between optical rotatory power and other determinations of derris and cube roots. Second set of samples*

Sample no.	Material	Rotenone by crystallization from CCl₄	Acetone extraction		"Rotenone" from optical rotatory power	Benzene extraction		"Rotenone" from optical rotatory power	"Rotenone" from optical rotatory power of acetone and benzene extracts	"Toxic value" from rotenone and total benzene extractives
			Total extractives	Optical rotatory power [1]		Total extractives	Optical rotatory power [1]			
		Percent	*Per-cent*	*Degrees Ventzke*	*Percent*	*Per-cent*	*Degrees Ventzke*	*Percent*	*Percent*	*Percent*
2710	Derris root	3.3	18.7	[1]−1.8	3.0	15.6	−9.7	7.3	10.9	9.4
2227-R-344	do	6.5	22.0	[2]−5.0	8.2	19.0	−14.0	10.5	12.4	12.8
2793	do	2.9	20.1	[2]−1.4	2.3	17.6	−10.2	7.6	12.1	10.2
2768	do	4.9	19.6	−3.9	6.4	17.8	−12.0	9.0	11.2	11.4
2752	do	5.6	18.9	[2]−2.6	4.3	15.5	−11.7	8.8	12.5	10.6
2708	Cube root	5.2	18.3	−6.6	10.9	17.8	[2]−14.2	10.6	10.4	10.2
2709	do	3.7	16.5	−5.5	9.1	16.4	[2]−12.8	9.6	10.0	8.8
2711	do	1.2	10.7	−2.8	4.6	9.8	[2]−6.0	4.5	4.4	4.6
2779	do	1.7	13.1	[2]−4.6	7.6	12.7	[2]−8.6	6.4	5.5	6.1
2794	do	5.3	18.2	−6.6	10.9	17.2	−14.2	10.6	10.4	10.1
998	Derris root	0	15.2	[2]+2.0	(³)	13.4	−4.7	3.5	9.2	6.7
999	do	0	13.2	[3]+1.6	(³)	11.5	−4.2	3.1	7.9	5.8
1000	do	0	14.4	[2]+1.8	(³)	13.0	−4.8	3.6	9.0	6.5
2288	do	0	18.6	+3.5	(³)	16.2	−5.2	3.9	12.0	8.1
2843	do	0	16.2	[2]+2.4	(³)	13.4	−4.3	3.2	9.2	6.7

[1] 100 cc○10 g of plant material, calculated for 2-dm tube.
[2] Determinations were made in 1-dm tube.
[3] Not calculable.

The values for optical rotatory power and equivalent "rotenone," together with the rotenone content by extraction, and total acetone and benzene extractives, are given in table 2.   The figures for "toxic value" from rotenone and total extractives are also included to give some comparison between the optical rotatory power and the insecticidal activity in lieu of actual toxicity tests.

<center>DISCUSSION</center>

It will be noted that in the derris roots containing rotenone the sets of values calculated by the three optical rotation methods are quite different.   The acetone values are comparable with the actual rotenone content, but the other figures are markedly higher.   On the other hand, the values for the cube roots obtained by the three methods are in fair agreement.   This tendency was also noted in the first set of cube samples.   The agreement might be explained by assuming that all the optically active components are similar to rotenone in exhibiting approximately double the rotatory power in benzene that they do in acetone.   In such a case the optically active components of practically all the cube samples would be similar to rotenone in this respect, and hence perhaps very closely related to rotenone.   Further, it would follow that most of the derris samples contain materials that are quite unlike rotenone in their optical rotatory relations.

It will be noted that the agreement between the "rotenone" calculated from the combined optical rotatory powers of acetone and benzene extracts and the "toxic value" from rotenone and total extractives is good, indicating that the former values would agree with the actual toxicity to houseflies.   For the derris roots containing no rotenone the figures for toxic value are somewhat lower than the combined optical rotation values, as was also true of the derris sample containing no rotenone in the first set of roots.

Acetone extracts of the derris samples containing no rotenone were dextrorotatory although the benzene extracts were levorotatory, as were the derris containing no rotenone (no. 401) in the first set of roots.   In these samples the combined values from benzene and acetone extracts were markedly higher than those from benzene alone.   It is obvious that these samples contain some dextrorotatory substances.   It may be supposed that they also contain some levorotatory material which, like rotenone, has a higher activity in benzene, and thus in the benzene extracts masks the dextrorotatory material.   Dextrorotatory components may also be present in other samples of root, particularly those showing poor agreement between the three sets of optical rotation values, but they would be masked in both acetone and benzene by the strong levorotation of the rotenone present.   Rowaan (*12*) actually obtained some values by benzene rotation which were lower than the rotenone by crystallization.   In no case have the writer's benzene values been below the rotenone content, although the acetone values for a few of the derris samples were lower.

Rowaan has suggested that toxicarol, which he believes to be dextrorotatory, may account in part for these lower values.   This might also contribute to the dextrorotation of the writer's "no rotenone" samples in acetone, as well as account for some of the disagreement between the three sets of values for some of the samples.   It might

therefore be conjectured that the samples of cube root studied contained little or no toxicarol while most of the samples of derris contained appreciable amounts.

A comparison of the rotenone values obtained by extraction with those obtained by optical rotation, particularly of benzene extracts, shows that in many of the samples there must be materials in addition to rotenone which possess a higher levorotation in benzene at least.

SUMMARY AND CONCLUSIONS

Values for "rotenone" equivalent to the combined optical rotatory powers of both acetone and benzene extracts of derris and cube roots gave an approximate measure of the insecticidal effectiveness of these materials to houseflies. Values calculated from the rotation of benzene extracts did not agree with toxicity so well as did the combined values, and in about half the samples results derived from the optical activity of acetone extracts were widely different from the toxicity values.

Since a method has already been proposed for calculating the approximate toxic value to houseflies of derris and cube roots based on the rotenone and total extractive contents, which is both simpler and less open to question, the use of optical rotatory power cannot be recommended as a means of evaluation.

From the chemical standpoint the results indicate that optically active constituents other than rotenone and deguelin were probably present in the samples of derris and cube tested. Dextrorotatory materials were undoubtedly present in the samples of derris root containing no rotenone, and possibly in other samples. The use of optical rotation should prove of considerable value in further chemical study of the components of extracts of derris and cube roots.

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RELATIVE TOXIC ACTION OF PHENOL AND PHENYL MERCAPTAN, WHEN THE GOLDFISH IS USED AS THE TEST ANIMAL [1]

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INTRODUCTION

In the course of toxicological studies an investigation was made of the toxicity of some of the simpler aromatic substances, beginning with phenol. Since it is known that the replacement of the oxygen atom in the molecule by sulphur frequently increases toxicity, phenyl mercaptan (thiophenol) was selected for a comparative study. The relationship of the two compounds is shown by their structural formulas, $C_6H_5.OH$ and $C_6H_5.SH$.

The boiling point of the sample of phenol used was 183° C., and that of the phenyl mercaptan was 170°. The samples were, therefore, of a high state of purity.

Since rotenone has been used as a standard of comparison in previous studies of a similar nature (6, 7),[2] it was again used as such, and tests were made to establish the resistance of goldfish to this substance.

EXPERIMENTAL PROCEDURE

The method of making the toxicity tests is essentially the same as that previously described (3). Goldfish of a single lot, weighing from 3 to 5 g, were used, and a constant temperature of 27° C. was maintained.

Phenol is readily soluble in water to such an extent that aqueous stock solutions could be used. Phenyl mercaptan, however, because of its insolubility, was first dissolved in acetone, and aliquot portions were used to give better dispersion in the test solutions.

Unfortunately, phenyl mercaptan is not completely soluble at most of the concentrations used, as evidenced by the appearance of some minute oily droplets. In the very lowest concentrations these seemed to disappear after a short while, but in others they were replaced by crystals, which proved to be phenyl disulphide. When exposed to oxidizing agents phenyl mercaptan has a tendency to throw off hydrogen and form this compound. This reaction progressed to some extent under the conditions of the tests, but it was found to be relatively slow in the low concentrations if the aliquots were dispersed in the test solutions without undue aeration. In a separate test at a concentration of 10 mg per liter the crystals began to form in about half an hour, but the change was not complete in 4 hours. This solution, as well as those of lesser concentration, became faintly opalescent in 3 to 4 hours. At a concentration of 2 mg per liter there was no apparent opalescence. The toxic action, however, proceeded in an

orderly fashion, both as to frequency distributions of survival times at each concentration and as to variation of mean survival time with variation in concentration, even in the range of markedly opalescent solutions. It would appear, therefore, that the toxic effect of the substance is the same whether collodial or in true solution and that the toxic action is not appreciably affected by the partial oxidation of phenyl mercaptan to phenyl disulphide.

Although the concentrations of phenol used were relatively high, the changes in hydrogen-ion concentration caused by its use were well within the range of the variation tolerated by most fishes. Waters having an acidity as high as pH 4.0 may support a good, mixed fish fauna (2). A solution of phenol of the highest concentration used has a pH value of 5.7. Wiebe (9) has reported that goldfish tolerated a sudden change from water of pH 7.5 to water of pH 6.2. The higher value approximates the average for the city water to which the fish used in this study were acclimated and which was used in making the solutions, and the lower value is that of a solution of 0.28 g of phenol per liter. It would appear, then, that the variation in hydrogen-ion concentration did not introduce a disturbing factor.

DISCUSSION OF TOXIC ACTION

The toxic action of phenol on goldfish is quite different from that of rotenone (1, 3, 4). The effect of rotenone is one of suffocation through the medium of the destruction of the gill epithelium, and it is a gradual one, there being apparent only a slowly increasing sluggishness. Also there is a comparatively large time tolerance; that is, the survival time required for lethal action at high concentrations is long. In the case of phenol, on the other hand, the fish are strongly affected at once, as shown by a frantic dashing about, and later movements are characterized by spasmodic twitchings. Respiratory movements are slightly increased and become irregular and forced. The fish show no evidence of oxygen hunger as in the case of rotenone poisoning. There is practically no time tolerance. The higher concentrations exert an obviously strong paralytic action, as evidenced by rigid, outstanding fins and locked jaws. This is the type of action produced by nicotine and anabasine (5).

In rotenone poisoning the last stage of the physiological action just preceding death, after irritability is lost, is a relatively long one, but in phenol poisoning it is short. In the test to confirm death (dipping the fish into 1:3 hydrochloric acid), with phenol some of the individuals were still capable of muscular movement. Thus, fish killed with phenol retain this ability nearer to the time of death than those killed with rotenone.

Phenyl mercaptan exerts a rapid toxic action like that of phenol, but its insolubility at the higher concentrations required in the tests under the present conditions precludes a definite statement regarding a time tolerance. Despite this, however, there is indicated a time tolerance much smaller than that of rotenone.

TREATMENT OF DATA

Other differences in the action of phenol were soon noted, and because of these peculiarities a study of the frequency distributions was made. As a result of the findings the mathematical treatment

of the data differs from that ordinarily employed in respect to the form of average selected as defining the position of each frequency distribution for the observed survival times. In the tests with rotenone the distributions were nearly normal, the mode corresponding closely with the arithmetic mean. The number of individuals used in a test was insufficient to place the position of the median with stability; however, the average of the middle third closely approximated the average of the whole. Therefore, the arithmetic mean gave the most probable value satisfactorily. On the other hand, in the tests with phenol the frequency distributions were markedly skewed, the degree of skewness increasing rapidly with the decrease in the concentration. Apparently this is due to a strong tendency of individuals to exhibit an increasing resistance to phenol beyond a certain point in the distribution. Thus, a few widely outlying observations in some cases pulled the arithmetic mean far to the right of the mode, and therefore, without the use of a large number of fishes—many more than were permitted by the conditions of the study—the value of this mean was very unstable. The degree of dispersion and the percentage of individuals exhibiting this effect become greater with decrease in concentration, at least to the lowest concentration used. This type of action was also found to be exhibited by nicotine and anabasine, and, since all three compounds exert an obviously strong paralytic action at the stronger concentrations, an asymmetrical distribution of such a high degree of skewness may be common to all compounds of this type of toxic action.

Thus it is seen that the assumption, which is implied by the use of the arithmetic mean, that plus and minus deviations from the mean are equally probable, cannot be justified in this type of toxic action. Frequently at the lower concentrations the longer survival times are more than double the mean. The median is also an unstable value. The mode, although well defined at the higher concentrations, is not so well defined at the lower concentrations where the degree of dispersion becomes great, especially without great increase in the number of observations beyond those permitted in these studies.

The harmonic mean would have the desired property of a lowered relative effect of a few long survival times. It would have, moreover, an easily understandable meaning, for it would simply give, instead of the average survival time at a given concentration, the survival time corresponding to the average velocity of fatality. There is, however, an important objection to its use. Occasionally an individual was found to die much sooner than his companions in a test. This was not due to an obviously poor physical condition, for special precautions were taken to eliminate as far as possible this source of error. Since such an individual appears only occasionally, its effect on the instability of the harmonic mean is considerable. Thus the use of this mean has a disadvantage which it would be desirable to avoid.

In these toxicity tests the magnitudes of the deviations in the various distributions appear to be dependent in some degree on the magnitude of the average. Moreover, the individual observations appear to fall into a geometric rather than an arithmetic series. Therefore, it may be logical to measure variations by their ratio to, rather than their difference from, the average. In this case the geometric mean is the natural average to use.

The study of a number of distributions shows that the frequency curve, if plotted to the logarithms of survival times instead of the observed times, becomes much more symmetrical around the mean, which approaches more closely to the mode (a geometric mode) and therefore the median. For these reasons, in tests with compounds giving such skewed distributions as do the phenols, it appears that the geometric mean defines best the position of the frequency distribution and so will be used in this study. Phenyl mercaptan does not ex-
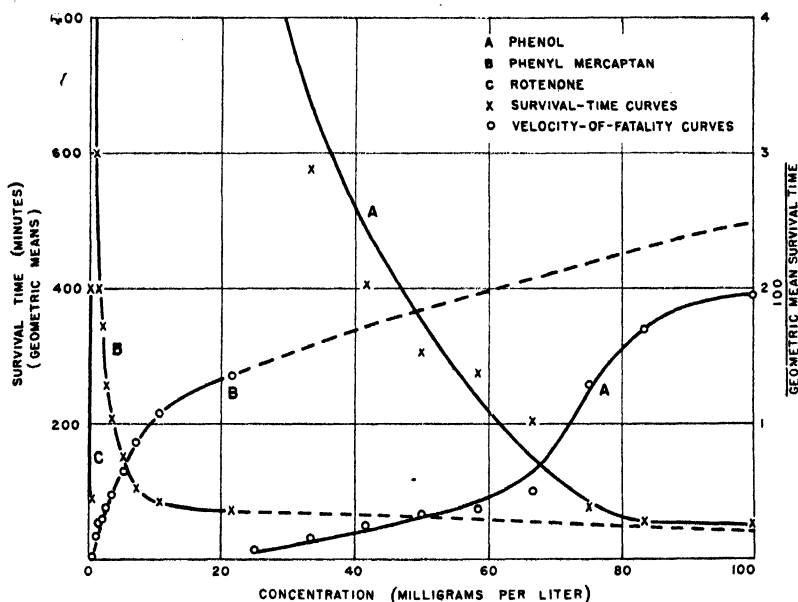
FIGURE 1.—Toxicity curves of phenol, phenyl mercaptan, and rotenone. Rotenone is comparatively so toxic that the features of its survival-curve time cannot be brought out graphically at the scale required to show those of the other compounds.

hibit the degree of skewness that phenol does, its frequency distributions resembling those of rotenone in having but a moderate asymmetry. Since the toxicity of these two compounds is to be compared with that of phenol, however, the results obtained with them are to be treated in the same way.

RESULTS

The toxicological data are given in table 1. The survival-time curves and the velocity-of-fatality curves which are plotted from these data are given in figure 1.

TABLE 1.—*Toxicity of phenol, phenyl mercaptan, and rotenone to goldfish at 27.0° ± 0.2° C.*

Concentration (milligrams per liter)	Fishes used	Mean length of fishes	Mean weight of fishes [1]	Mean survival time [2] Arithmetic	Mean survival time [2] Geometric	100÷geometric mean survival time
ROTENONE						
	Number	*Millimeters*	*Grams*	*Minutes*	*Minutes*	
0.500	14	46	2.9	92± 3	90 1.03	1.11
.300	17	47	3.1	97± 3	96 1.01	1.04
.200	12	46	2.9	106± 5	103 ×1.04	.971
.150	8	53	4.4	138±10	134 +1.07	.74
.100	22	50	3.6	170±11	154 1.06	.649
.0750	13	55	5.0	215±16	200 1.07	.500
.0500	10	(3)	(3)	> [4] 400	> [4] 400	< .250
PHENOL						
2,380	4	56	5.3	6	6	17
820	4	53	4.5	9	9	11
311	4	52	4.3	46± 5	43 1.16	2.33
207	5	55	5.0	39± 3	38 1.10	2.63
124	6	55	4.9	48± 6	45 1.11	2.22
99.8	13	47	3.1	53± 3	51 1.06	1.96
83.2	22	49	3.5	84± 3	59 ×1.05	1.69
74.9	8	52	4.3	116± 29	78 1.23	1.28
66.6	10	54	4.8	231± 26	202 ÷1.10	.50
58.3	7	56	5.3	315± 43	273 1.16	.37
50.0	18	54	4.7	478± 83	306 1.16	.33
41.6	12	56	5.2	624±121	406 1.25	.25
33.3	12	54	4.8	702± 67	577 1.16	.17
25.0	10	(3)	(3)	[5] 1,420	> [5] 1,260	<.08
PHENYL MERCAPTAN						
216.0	2	45	2.8	25± 2	25 1.09	4.00
21.6	2	52	4.3	75± 9	74 1.12	1.35
10.8	7	57	5.6	86± 4	85 1.04	1.18
7.19	9	52	4.3	119± 4	117 ×1.04	.855
5.39	10	53	4.6	154± 6	152 ÷1.04	.658
3.59	13	54	4.9	211± 7	208 1.03	.481
2.70	12	54	4.9	261±11	257 1.04	.389
2.16	10	56	5.3	349±14	344 1.04	.291
1.62	11	54	4.9	> [6] 400	> [6] 400	< [6] .250
1.08	9	48	3.4	> [6] 600	> [6] 600	< [6] .167
.54	10	(3)	(3)	> [7] 4,000	> [7] 4,000	< [7].025

[1] Estimated from length, which measurement excludes the tail.
[2] Errors are probable errors of the means. At the first 2 concentrations of phenol a probable error, if calculated, would have no significance at such short survival times.
[3] Fishes not measured or weighed but of same approximate size.
[4] 1 fish dead in 210 minutes; 1 near death and 8 fishes not near death in 400 minutes, when test was stopped.
[5] 4 fishes died in 850 minutes; 6 still alive after 30 hours, when test was stopped.
[6] Slightly low; temperature not controlled satisfactorily.
[7] Not killed in 66 hours, when test was stopped.

QUANTITATIVE COMPARISON OF RESULTS

A striking difference in the toxic action of phenol and phenyl mercaptan is at once apparent from an inspection of the velocity-of-fatality curves. That of phenol has a sigmoid curvature, signifying that the initial as well as the final acceleration with increase in concentration is relatively slow, whereas that of phenyl mercaptan apparently lacks the initial upward concavity. This is again suggestive of the types of curves obtained with nicotine and rotenone, respectively. Whether this is due to the comparatively large differences in toxicity or to the particular types of action, especially as regards complexity of physiological action, remains to be determined.

To attempt to compare quantitatively such different types throughout their course of action would be meaningless, especially as to thresholds of toxicity or the theoretical velocity of fatality of Powers (8). The threshold of phenol cannot be approximated. Under the conditions of the tests, however, the threshold concentration of rotenone is about 0.04 mg per liter, and that of phenyl mercaptan about 0.4 mg per liter. It may be said, therefore, that phenyl mercaptan becomes toxic at much higher concentrations than rotenone (about 10 times as high) whereas phenol becomes significantly toxic (lethal within 3 days) at still higher concentrations than phenyl mercaptan (of the order of 40 times as high). This appears to be a fair comparison from the standpoint of concentration alone.

The insolubility of phenyl mercaptan at the highest concentrations necessary with this particular lot of fish doubtless affects the shape of its curve, and, therefore, any quantitative comparison at this range of the time tolerance would have no general significance.

The compounds may properly be compared, however, at their regions of most efficient action. In these studies this action is a function of concentration and survival time, and a criterion providing such a quantitative comparison has been suggested by the author (6). This criterion is the minimum product of concentration and survival time as determined from the survival-time curve, and toxicity under these conditions may be defined as the reciprocal of this product. In the case of rotenone its curve must first be redrawn on an expanded scale. The comparative data are given in table 2. The minimum product of concentration and time is designated by $(ct)_m$; its approximate coordinates, c_m and t_m, are given so that the region of the curve fulfilling this condition can be located readily.

TABLE 2.—*Relative toxicity at 27° C. of phenol, phenyl mercaptan, and rotenone*

Compound	c_m	t_m	$(ct)_m$	Toxicity, $1/(ct)_m$	Relative toxicity according to $(ct)_m$
	Milligrams per liter	*Minutes*	*Gram-minutes per liter*	*Liters per gram per minute*	
Rotenone	0.075	200	0.0150	66.7	1.0
Phenol	85.0	55	4.68	.214	.0032
Phenyl mercaptan	2.00	345	.690	1.45	.022

From these data it is seen that, according to this criterion, phenyl mercaptan is 6.9 times as toxic as phenol but only 0.022 as toxic as rotenone.

SUMMARY

A study has been made of the toxicity of phenol and phenyl mercaptan, and the results have been compared with each other and with those obtained with rotenone. Goldfishes weighing 3 to 5 g each, and of the same lot, were used as the test animals. The observations were made at a constant temperature of 27° C.

The phenolic solutions used were not so acid that the change in hydrogen-ion concentration was a factor to be considered.

Phenol, like nicotine and anabasine, gives markedly skewed frequency distributions, in contradistinction to phenyl mercaptan and rotenone. The geometric mean defines best the position of the distribution in the case of phenol. It differs but little from the arithmetic mean in the case of phenyl mercaptan and rotenone.

Phenyl mercaptan becomes toxic at much higher concentrations than rotenone (about 10 times as high), whereas phenol becomes significantly toxic (lethal within 3 days) at still higher concentrations than phenyl mercaptan (of the order of 40 times as high). Under the conditions of this study the threshold concentration of rotenone is about 0.04 mg per liter and that of phenyl mercaptan is about 0.4 mg per liter. That of phenol, however, cannot be approximated for comparison, since the initial acceleration of its velocity of fatality with increase in concentration is relatively slow.

Phenol has a much more rapid toxic action than rotenone; its effect is immediately apparent, and there is practically no time tolerance at the higher concentrations. Phenyl mercaptan, although not wholly soluble at the higher concentrations required under the foregoing conditions, also has a rapid toxic action.

All the compounds may be significantly compared quantitatively only at their regions of greatest toxic power, of which the minimum product of concentration and survival time is a measure. According to this criterion phenyl mercaptan is seven times as toxic as phenol but only one-fiftieth as toxic as rotenone.

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THE PHYSIOLOGY OF RHIZOPUS ORYZAE [1]

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INTRODUCTION

Several workers have reported the occurrence of lactic acid among the products of the metabolism of glucose by fungi. A survey of the literature and of the work done in this Division on the production of *d*-lactic acid by various species of *Rhizopus* has been made the subject of another paper (*8*).[2] This paper presents a more detailed discussion of the physiology of *Rhizopus oryzae* Went and Geerligs 395 in connection with the production of *d*-lactic acid and fumaric acid.

DESCRIPTION OF ORGANISM

When grown on sterile moistened white bread, this culture makes a dense felt 5 to 7 cm thick of white stolons, at the nodes of which are borne clusters of brown much-branched rhizoids and clusters of 2 to 10 sporangiophores. Sporangiophores are 200μ to 6 mm long by 10μ to 25μ thick. They are brown, erect, branched or unbranched, with smooth walls terminating in a swelling, the apophysis, which with the sporangium forms a sphere. Sporangia are 100μ to 300μ in diameter. They are dark yellowish brown, and their walls are roughened with minute warty protuberances. The columella is spheric to hemispheric, sometimes collapsing, brown, and 70μ to 150μ in diameter. Sporangiospores are angular or irregular in shape, minutely ridged longitudinally, colorless or brown to black in mass, and 5μ to 12μ in diameter. Chlamydospores are elliptic to spheric, or cylindrical, 18μ to 35μ by 10μ to 20μ.

Culture no. 395 in the collection of this Division, of unknown origin, was compared with descriptions of *Rhizopus oryzae* and with numerous cultures received under this name. It is morphologically similar to a culture of *Rhizopus oryzae* supplied by Dr. A. J. Kluyver, University of Delft, Netherlands.

MATERIALS AND METHODS

For storage purposes, cultures were maintained on beer and wort agar slants at 7° C. In order to obtain spores sufficient for the inoculation of a large number of flasks, the organism was transferred to sterile moistened bread. After 4 or 5 days at 30°, this was covered with a felt of stolons, and sporulation was abundant. Some of this mass of stolons and sporangiospores was transferred to a quantity of sterile water. The spores were liberated but vigorous agitation of the suspension, and the stolon mass was then removed by a sterile hook. Counts of the number of spores per cubic centimeter were made with the aid of a hemocytometer, and the suspension was diluted with sterile water to give the desired number of spores per unit volume. One

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cubic centimeter of inoculum was pipetted aseptically into each of the experimental flasks. Since it was found that uniform results could be obtained throughout the range of 12.5 to 100 million spores per flask, some variation in the number of spores per culture was allowed. At the time of inoculation, 5 g of sterile $CaCO_3$ was added to the nutrient solution, unless otherwise stated. For experimental purposes cultures were grown in triplicate in pyrex Erlenmeyer flasks of 200 cc capacity. Ordinarily, the volume of nutrient used was 75 cc per flask. Nutrient solutions were sterilized in an autoclave at 15 pounds steam pressure for 15 minutes. Experimental cultures were maintained at 30° unless otherwise stated.

Throughout most of this work, a commercial grade of glucose monohydrate was employed as the sole carbon source. In one series of cultures, a chemically pure grade of glucose was used. In all cases, the glucose is expressed in terms of anhydrous glucose as determined by the copper reduction method of Shaffer and Hartmann (7). Calcium was determined as calcium oxalate by permanganate titration. Fumaric acid was determined by the method described by Hahn and Haarmann (2), and lactic acid was determined by the method reported by Friedemann and Graeser (1).

RESULTS

COMPARISON OF RHIZOPUS ORYZAE CULTURES GROWN AT 30° AND 40° C.

Cultures were incubated for 15 days, some at 30° and some at 40° C. Data presented in table 1 indicate that cultures maintained at 40° grew more rapidly and contained the larger quantity of calcium in solution at the end of 15 days. However, at the higher temperature the amount of soluble calcium accounted for as the *d*-lactate was less, expressed as percentage of soluble calcium, than in the cultures grown at the lower temperature. Moreover, the total lactic acid produced was less in the culture grown at the higher temperature than in the one grown at the lower temperature. Although no fumaric acid was found in this series of cultures when grown at 30°, a considerable quantity was produced at the higher temperature.

TABLE 1.—*Metabolism of Rhizopus oryzae at 30° and 40° C.*

[75 cc 15-percent glucose; 0.25 g $MgSO_4.7H_2O$, 0.3 g KH_2PO_4, and 2.88 g NH_4NO_3 per liter; 5 g $CaCO_3$ per flask. Duration, 15 days]

Temperature °C.	Weight of mycelium	Glucose consumed	Calcium dissolved			*d*-Lactic acid		Fumaric acid	
			Total	Due to *d*-lactic acid	Due to fumaric acid	Weight	Weight yield [1]	Weight	Weight yield [1]
	Grams	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
30	0.352	6.4	0.86	[2] 105	0	4.07	63.5	0	0
40	.947	10.8	1.14	66	17.5	3.38	31.3	.575	5.3

[1] Grams of acid produced divided by grams of glucose consumed.
[2] Approximate.

DURATION OF CULTURE

In order to find when fumaric acid appeared in the culture, triplicate cultures were harvested on the fifth, ninth, thirteenth, seventeenth,

and twenty-first days after inoculation. Data presented in table 2 show rather slow growth and little acid production up to the ninth day, after which the rates of growth and fermentation rose rapidly. The beginning of this period of increased activity immediately preceded the formation of stolons and the onset of sporulation. The observation that fumaric acid appeared at the age of approximately 2 weeks is in general agreement with the work of Kanel (*3*), who reported that although no fumaric acid was present in young cultures it was found in older cultures.

TABLE 2.—*Influence of duration of culture*

[75 cc 15 percent glucose; 0.25 g $MgSO_4 \cdot 7H_2O$, 0.3 g KH_2PO_4, and 2.88 g NH_4NO_3 per liter; 5 g $CaCO_3$ per flask. Temperature, 30° C.]

Age	Weight of mycelium	Glucose consumed	Calcium dissolved			d-Lactic acid		Fumaric acid	
			Total	Due to d-lactic acid	Due to fumaric acid	Weight	Weight yield [1]	Weight	Weight yield [1]
Days	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
5	0.040	1.3	0.067			0.060			
9	.039	2.0	.24	87	0	.93	46.5	0	0
13	.220	6.3	.85	88	0	3.36	53.3	0	0
17	.889	10.5	1.28	78	8.7	4.50	42.9	.314	3.0
21	1.032	11.2	1.44	72	13.9	4.66	41.6	.564	5.0

[1] Grams of acid produced divided by grams of glucose consumed.

Data presented in table 2 show that *Rhizopus oryzae* did not utilize the *d*-lactic acid during the later stages of the fermentation. Further experiments have given the same result, even though the cultures were incubated for 49 days and no glucose was present in the flasks during the last 20 days of this period.

NUTRITION

EFFECT OF VARYING THE GLUCOSE CONCENTRATION OF THE NUTRIENT MEDIUM

The glucose content of the nutrient solution was increased from 10 to 30 percent by additions of approximately 5 percent. Data presented in table 3 indicate that a glucose concentration of 15 percent was most favorable, as measured by the weights of the mycelia, the consumption of glucose, and the formation of lactic acid. It is worthy of note that no fumaric acid was found in the culture solutions having the highest original glucose content.

TABLE 3.—*Effect of varying the glucose concentration*

[75 cc nutrient solution contained 0.25 g $MgSO_4 \cdot 7H_2O$, 0.3 g KH_2PO_4, and 1.44 g NH_4NO_3 per liter; 5 g $CaCO_3$ per flask. Duration, 14 days. Temperature, 30° C.]

Glucose concentration	Weight of mycelium	Glucose consumed	Calcium dissolved			d-Lactic acid		Fumaric acid	
			Total	Due to d-lactic acid	Due to fumaric acid	Weight	Weight yield [1]	Weight	Weight yield [1]
Percent	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
10.0	0.293	6.8	1.05	84.7	3.8	4.00	58.8	0.12	1.8
15.1	.324	8.5	1.26	87.4	3.5	4.96	58.4	.13	1.5
20.1	.266	7.6	1.06	86.6	3.2	4.14	54.5	.10	1.3
24.9	.044	3.0	.29	83.0	([2])	1.10	36.7	([2])	
30.0	.129	5.4	.49	88.0	0	1.95	36.1	0	0

[1] Grams of acid produced divided by grams of glucose consumed.   [2] Trace.

EFFECT OF VARYING THE KH₂PO₄ CONCENTRATION OF THE NUTRIENT MEDIUM

Cultures were made which contained 0.15, 0.3, 0.6, and 1.2 g of KH_2PO_4 per liter. Approximately 15 percent of glucose, 0.25 g of $MgSO_4.7H_2O$ per liter, and 1.44 g of NH_4NO_3 per liter were used in each. Data presented in table 4 show that although the differences were not great, the cultures with the two highest quantities of KH_2PO_4 were most active in the consumption of glucose and the formation of *d*-lactic acid. At these two concentrations, the percentage of the soluble calcium accounted for as calcium *d*-lactate was also greatest.

TABLE 4.—*Effect of varying the KH_4PO_4 content of the nutrient medium*

[75 cc nutrient solution contained approximately 15 percent, glucose; 0.25 g $MgSO_4.7H_2O$ and 1.44 g NH_4 NO_3 per liter; 5 g $CaCO_3$ per flask. Duration, 15 days. Temperature, 30° C.]

KH₂PO₄ per liter (grams)	Weight of mycelium	Glucose consumed [2]	Calcium dissolved			d-Lactic acid		Fumaric acid	
			Total	Due to d-lactic acid	Due to fumaric acid	Weight	Weight yield [1][2]	Weight	Weight yield [1][2]
Grams	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
0.15	0.397	8.1	1.24	78.5	8.1	4.38	54.1	0.28	3.5
.30	.416	8.2	1.21	81.7	7.4	4.44	54.1	.26	3.2
.60	.419	9.2	1.41	86.4	7.1	5.47	59.5	.28	3.0
1.20	.372	8.9	1.36	85.5	4.4	5.25	59.0	.18	2.0

[1] Grams of acid produced divided by grams of glucose consumed.　　　[2] Approximate.

EFFECT OF VARYING THE NH₄NO₃ CONCENTRATION OF THE NUTRIENT MEDIUM

Nitrogen was supplied to a series of cultures as NH_4NO_3 in the quantities of 0.75, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, and 12.0 g of NH_4NO_3 per liter. Data presented in table 5 show that the optimum range of concentration of NH_4NO_3 for growth as measured by the weight of the mycelia was 3.0 to 6.0 g per liter. The range most favorable for the formation of *d*-lactic acid was 1.5 to 6 g per liter. However, better yields of lactic acid, based on the glucose consumed, were obtained at the lowest and highest concentrations than at the concentrations which were optimal for the growth of the fungus and the production of *d*-lactic acid in quantity. Table 5 also shows that at the higher concentrations of NH_4NO_3 (7.5 to 12 g per liter) fumaric acid was not found. At all the lower concentrations fumaric acid was found.

TABLE 5.—*Effect of varying the NH_4NO_3 concentration of the nutrient medium*

[75 cc 13.2-percent glucose nutrient solution contained 0.25 g $MgSO_4.7H_2O$ and 0.3 g KH_2PO_4 per liter; 5 g $CaCO_3$ per flask. Duration, 17 days. Temperature, 30° C.]

NH₄NO₃ per liter (grams)	Weight of mycelium	Glucose consumed	Calcium dissolved			d-Lactic acid		Fumaric acid	
			Total	Due to d-lactic acid	Due to fumaric acid	Weight	Weight yield [1]	Weight	Weight yield [1]
Grams	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
0.75	0.134	5.6	0.99	88.5	5.3	3.94	70.4	0.150	2.7
1.5	.290	6.9	1.08	88.8	4.4	4.34	62.9	.140	2.0
3.0	.656	8.0	1.17	82.0	4.3	4.49	56.1	.155	1.9
4.5	.744	8.1	1.11	86.5	4.2	4.29	53.0	.135	1.7
6.0	.767	7.6	1.07	87.5	(2)	4.22	55.5	(2)	
7.5	.233	4.8	.78	88.4	0	3.09	64.4	0	0
9.0	.145	3.3	.58	89.5	0	2.35	71.2	0	0
12.0	.214	2.2	.67	80.5	0	2.42	[3] 110.0	0	0

[1] Grams of acid produced divided by grams of glucose consumed.　　　[2] Trace.　　　[3] Approximate.

USE OF VARIOUS NITROGEN SOURCES BY RHIZOPUS ORYZAE

NaNO$_3$, NaNO$_2$, (NH$_4$)$_2$SO$_4$, NH$_4$Cl, d, l-alanine, d-glutamic acid, glycine, and peptone were supplied, each as the sole source of nitrogen, in quantities equivalent to 0.5 g of nitrogen per liter. NH$_4$NO$_3$ and (inadvertently) urea were supplied in quantities equivalent to 1 g of nitrogen per liter. It had been found that not 1 of about 50 cultures studied was able to utilize nitrate nitrogen. The ammonium nitrogen present in the NH$_4$NO$_3$ supplied was equivalent to the total nitrogen supplied in the other nitrogen sources. When NaNO$_3$ was supplied no growth occurred; when NaNO$_2$ was supplied, scant growth averaging 0.026 g per flask, occurred. Data presented in table 6 show that all the other sources of nitrogen supplied were fairly satisfactory. It is significant that this organism was able to utilize ammonium and amino nitrogen, but not nitrate nitrogen, and it utilized nitrite nitrogen very poorly.

TABLE 6.—*Effect of different nitrogen sources on the metabolism of Rhizopus oryzae*

[75 cc 15.1-percent glucose nutrient solution contained 0.25 g MgSO$_4$·7H$_2$O and 0.3 g KH$_2$PO$_4$ per liter; 5g CaCO$_3$ per flask. Duration, 14 days. Temperature, 30° C.]

Nitrogen source		Weight of mycelium	Glucose consumed	Calcium dissolved			d-Lactic acid		Fumaric acid	
Formula or name	Quantity per liter			Total	Due to d-lactic acid	Due to fumaric acid	Weight	Weight yield [1]	Weight	Weight yield [1]
	Grams	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
NaNO$_3$	3.00	0.000	0.0	0.00	0.0	0.0	0.00	0.0	0.000	0.0
NaNO$_2$	2.56	.026								
NH$_4$NO$_3$	2.88	.596	8.1	1.05	86.0	3.2	4.03	49.8	.099	1.2
(NH$_4$)$_2$SO$_4$	2.36	.301	6.2	.79	95.0	3.5	3.36	54.2	.082	1.3
NH$_4$Cl	1.98	.370	7.2	1.05	87.5		4.13	57.4		
Urea	2.14	.613	8.6	1.24	87.5	6.0	5.44	63.3	.217	2.5
d, l-alanine	3.30	.350	8.3	1.26	86.0	8.6	4.87	58.7	.312	3.8
d-glutamic acid	5.46	.356	7.1	1.08	83.0	6.6	4.05	57.0	.206	2.9
Glycine	2.67	.257	5.9	.87	86.0	7.2	3.37	57.1	.183	3.1
Peptone	3.24	.219	8.3	1.29	85.0	5.0	4.95	59.6	.189	2.3

[1] Grams of acid produced divided by grams of glucose consumed.

EFFECT ON METABOLISM OF RHIZOPUS ORYZAE OF THE ADDITION OF ZINC, IRON AND CHROMIUM SALTS TO THE NUTRIENT MEDIUM

Increased metabolic activity of various micro-organisms when salts of zinc, iron, or chromium were added to the nutrient media have been observed by various investigators (4, 5, 6). Accordingly, cultures were made to which 20 mg per liter of Zn, Fe, and Cr were added in the form of their salts as ZnSO$_4$·6H$_2$O, FeCl$_3$·6H$_2$O, and Cr(NO$_3$)$_3$· 6H$_2$O. The basic nutrient solution contained 14.2 percent of chemically pure glucose, 0.25 g of MgSO$_4$·7H$_2$O, 0.3 g of KH$_2$PO$_4$, and 2.88 g of NH$_4$NO$_3$ g per liter. Five-grams of CaCO$_3$ per flask was added to half the flasks of each group. There was no increased glucose consumption in the presence of any of the metallic ions when CaCO$_3$ was not supplied, but when it was supplied there was a marked increase in glucose consumption in the ZnSO$_4$ cultures, as well as an increase in the quantity of d-lactic acid produced. However, no difference resulting from the addition of CaCO$_3$ to the cultures which contained chromic or ferric ions was apparent. Accordingly, the effect of zinc on the metabolism of *Rhizopus oryzae* was further investigated. To a

series of cultures containing the same basic nutrient salt medium and 15.4 percent of commercial glucose, $ZnSO_4$ was added so that the zinc ion was present in quantities of 0.1, 1, 10, 20, 50, and 100 mg per liter. The data in table 7 show that the addition of $ZnSO_4$ to the culture medium exerted a marked influence on the growth of *R. oryzae* as measured by the weights of the mycelia, the consumption of glucose, and the production of *d*-lactic acid. The concentration of Zn of 10 to 100 mg per liter was most satisfactory for growth and glucose consumption. While the greatest production of *d*-lactic acid expressed in absolute quantity occurred at the Zn concentration of 10 mg per liter, when expressed as weight yields based on the glucose consumed, the percentage yields of *d*-lactic acid were progressively less as the Zn concentration increased.

TABLE 7.—*Effect of the addition of various quantities of $ZnSO_4$ to the nutrient medium* [

[75 cc 15.4-percent glucose nutrient medium contained 0.25 g $MgSO_4 \cdot 7H_2O$, 0.3 g KH_2PO_4, and 2.88 g NH_4NO_3 per liter; 5 g $CaCO_3$ per flask. Duration, 11 days. Temperature, 30° C.]

Zinc concentration per liter (milligrams)	Weight of mycelium	Glucose consumed	Calcium dissolved		*d*-Lactic acid	
			Total	Due to *d*-lactic acid	Weight	Weight yield [2]
	Grams	*Grams*	*Grams*	*Percent*	*Grams*	*Percent*
0.0	0.131	3.7	0.50	92	2.08	56.2
0.1	.189	3.5	.47	[3] 106	2.24	64.0
1.0	.316	4.6	.63	[3] 105	2.96	64.2
10.0	.830	8.3	.73	[3] 103	3.40	41.0
20.0	.807	7.8	.71	93	2.97	38.1
50.0	.804	8.3	.63	89	2.52	30.4
100.0	.669	7.7	.65	86	2.52	32.7

[1] No fumaric acid was produced.
[2] Grams of acid produced divided by grams of glucose consumed.
[3] Approximate.

FERMENTATION OF GLUCOSE BY FINISHED MATS OF RHIZOPUS ORYZAE UNDER DIFFERENT CONDITIONS OF NITROGEN NUTRITION

Data in table 2 show that fumaric acid is not produced during the first days of growth of the organism, and data in table 5 show that the production of fumaric acid may be suppressed by increasing the NH_4NO_3 concentration of the medium beyond 6 g per liter. With these facts in mind, cultures were made in which were used approximately 15 percent of glucose, and 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of KH_2PO_4, and 2.88 g of NH_4NO_3 per liter. After 17 days the solutions were drained from these cultures, and three series of triplicate cultures were established by the addition of 50 cc each of new culture solutions. One was similar to the original culture medium, another contained no nitrogen source, and in a third NH_4NO_3 was present at a concentration of 9 g per liter. Further harvests and replacements of solutions were made at 5-day intervals. Between the harvest of the culture medium and the replacement with a new nutrient solution the mycelial pads were floated on sterile distilled water for a short time to wash out any adherent glucose or metabolic products. Five grams of sterile $CaCO_3$ was added dry beneath the mats before the replacement solutions were supplied. The control cultures retained the original solutions throughout the 32-day period. Data

presented in table 8 show that high concentrations of nitrogen, even in the matured cultures, were unfavorable for the production of fumaric acid. Likewise, the nitrogen-starvation condition was unfavorable for fumaric-acid production. When the nitrogen supply was 2.86 g NH_4NO_3 per liter, the formation of fumaric acid depended on the maturity of the mycelium.

TABLE 8.—*Fermentation of glucose by finished mats of Rhizopus oryzae under varying conditions of nitrogen nutrition*

Nutrient solution contained 0.25 g $MgSO_4 \cdot 7H_2O$ and 0.3 g KH_2PO_4 per liter; 5.0 g $CaCO_3$ per flask. Temperature 30° C.]

NH_4NO_3 per liter (grams)	Age of mat	Glucose supplied	Glucose consumed	Calcium dissolved			d-Lactic acid		Fumaric acid		Weight of mycelium	Volume
				Total	Due to d-lactic acid	Due to fumaric acid	Weight	Weight yield [1]	Weight	Weight yield [1]		
	Days	Grams	Grams	Gr..ms	Percent	Percent	Grams	Percent	Grams	Percent	Grams	Cc
2.86	17	11.4	8.55	1.00	101	0.0	4.57	53.8	0.000	0.0	-------	75
0.00	22	7.5	6.2	.75	79.0	10.8	2.65	42.7	.236	3.8	-------	50
0.00	27	8.65	5.2	.63	76.0	8.4	2.17	41.7	.155	3.0	-------	50
0.00	32	7.5	3.6	.55	76.5	8.1	1.87	51.9	.129	3.6	0.885	50
2.86	22	7.5	6.7	.80	75.0	9.1	2.71	40.4	.211	3.1	-------	50
2.86	27	8.65	7.4	.72	57.0	27.9	1.84	24.9	.582	7.9	-------	50
2.86	32	7.5	7.0	.79	49.5	34.2	1.74	24.9	.780	11.1	2.631	50
9.00	22	7.5	7.3	.69	80.0	(2)	2.48	34.0	(2)		-------	50
9.00	27	8.65	8.3	.55	60.0	9.6	1.47	17.7	.154	1.9	-------	50
9.00	32	7.5	7.5	.47	66.0	.0	1.40	18.7	.000	.0	3.666	50
Control	32	11.4	11.4	1.23	84.5	7.3	4.69	41.1	.260	2.3	1.024	75

[1] Grams of acid produced divided by grams of glucose consumed.   [2] Trace.

DISCUSSION OF RESULTS

It will be noted that throughout this work the total acidity, as measured by calcium in solution, was never completely accounted for by lactic and fumaric acids. The preponderance of lactic acid in these solutions adds considerably to the difficulty of analysis, and at present the authors are not prepared to give an accurate and complete accounting for the total acidity. However, positive Denigès' tests for malic acid have been obtained on some of the solutions, and extremely small quantities of acetic acid have been isolated from certain liquors. It is probable that small amounts of succinic acid are also present. In addition to the five acids, d-lactic, fumaric, l-malic, acetic, and succinic, alcohol has been detected among the products of the glucose metabolism of *Rhizopus oryzae*.

The data presented in tables 1, 2, 3, 5, and 8 show that the production of fumaric acid varies and is governed to some extent by the cultural conditions and composition of the medium. Data in tables 2, 3, and 5 show that fumaric acid formation is suppressed by increased glucose concentration and by increased NH_4NO_3 concentration and is not found in cultures until after 13 days' incubation. Although table 1 shows no fumaric acid produced at 30° C., occasionally cultures of similar age were found throughout the course of this work in which fumaric acid was produced at 30°. However, in fumaric-acid production, cultures grown at 40° always showed marked superiority over those grown at 30°. The fact that fumaric acid was formed under similar conditions of nutrition in one series of cultures and not in another may have been due to differences in age or relative maturity of the mycelia.

In the section on the duration of culture it was reported that shortly after the thirteenth day the organism passes from the purely vegetative growth phase to the reproductive. This follows a sharp drop in the quantity of glucose remaining in the culture solution. It is doubtful whether sporulation is a reaction of starvation, for its occurrence corresponds with the greatest increase in weight of the mycelium. However, it should be borne in mind that the mycelium formed at this time is composed of stolons, which may be considered as accessory reproductive organs and probably play no part in the fermentation. Therefore, the pad of mycelium must be separated into two parts for interpretation of its metabolism. The first is the actively ferment-ing layer, which is in direct contact with the solution and which gives rise, after the fermentation has made considerable progress, to the second type of mycelium, the stolons. When $ZnSO_4$ is added to the nutrient medium, the formation of the second type of mycelium is hastened, so that sporulation occurs on the third day, when vigorous fermentation is just beginning.

That $ZnSO_4$ (20 mg Zn per liter) seriously alters the metabolism of the fungus is still further indicated by the way in which the yields of *d*-lactic acid decrease as the zinc concentration increases. At the lower concentration of zinc, sporulation is initiated later than at the concentration of 20 to 100 mg per liter.

The $CaCO_3$ used in the experiments reported here contained enough magnesium to support considerable fungus growth. Since, by adding $CaCO_3$, an undetermined quantity of magnesium was added to each culture, no investigation of the influence of magnesium on the metab-olism of *Rhizopus oryzae* was made.

SUMMARY

Factors influencing the production of dextro-lactic acid, fumaric acid, and the metabolism of *Rhizopus oryzae* have been investigated.

The temperature of 40° C. was more favorable for growth and glucose consumption than was that of 30°, but less favorable for *d*-lactic acid production.

The period of greatest activity in *d*-lactic acid production imme-diately preceded sporulation.

The glucose concentration most favorable for the production of *d*-lactic acid was approximately 15 percent.

Slightly greater yields of *d*-lactic acid were obtained when the KH_2PO_4 concentration was 0.6 or 1.2 g per liter than when the con-centrations were lower.

Fumaric acid production was suppressed in cultures containing more than 6 g of NH_4NO_3 per liter. A wide range of NH_4NO_3 con-centration, 1.5 to 6.0 g per liter, was favorable for *d*-lactic acid production.

Rhizopus oryzae readily utilized NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, urea, *d*-, *l*-alanine, *d*-glutamic acid, glycine, and peptone as nitrogen sources. $NaNO_2$ was an unsatisfactory nitrogen source, and when $NaNO_3$ was supplied as the sole source of nitrogen, no growth occurred.

In the presence of $CaCO_3$, $ZnSO_4$ exerted an influence on the metabolism of *Rhizopus oryzae*, the greatest quantity of *d*-lactic acid being produced at the concentration of 10 mg per liter of zinc.

Under favorable conditions of nitrogen metabolism, the formation of fumaric acid depended on the maturity of the mycelium.

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CHEMICAL COMPOSITION AND FERMENTATION STUDIES OF CITRON [1]

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INTRODUCTION

The citron, *Citrus medica* Linn., originally introduced into southern Europe from the Orient, is now grown in limited quantities in Puerto Rico, California, and Florida. When ripe the fruit resembles a giant lemon. It is normally picked while it is somewhat immature and the epicarp is still green. The essential oils in the fruit are oil of citron and oil of cedra, which are present in oil sacs in the skin (fig. 1). The thick mesocarp is used for preserving; the pulpy center containing the seeds is discarded.

The true citron should not be confused with the citron melon, a variety of *Citrullus vulgaris*, the common watermelon. The true citron is inedible in the raw state. In preparation for preserving it is usually halved and subjected to a prolonged fermentation in either sea water or a 5- to 10-percent salt brine. The fermentation removes the bitterness, clears the mesocarp, softens the tissues, and makes it possible for them to absorb high concentrations of sugar. Following the removal of salt from the peel, the preserving process consists essentially in gradually increasing the sugar content of the peel by immersion in a series of sugar sirups of increasing strength. After draining and drying, the peel is ready for market under the name of either preserved or candied citron.

Because of the paucity of published data on the fermentation and the chemical and nutritive properties of citron, a laboratory study involving these subjects seemed desirable. A review of the literature showed no analyses of fresh citron, few determinations of the mineral constituents of preserved citron, and no data at all on the vitamins. Furthermore, the findings of Chadefaux (3)[2] and Hollande and Chadefaux (8) on the fermentation of Corsican citron appeared not to have been substantiated by other investigators.

EXPERIMENTAL MATERIAL AND METHODS

Fresh unripe citrons were received biweekly from Puerto Rico. The fruit when stored kept perfectly for several weeks at a temperature of 35° F. Chemical analyses and vitamin A and C determinations were made on both the fresh and the preserved citrons. In order that comparable results might be obtained, analyses were made on the same lot of citrons before and after preserving.

FERMENTATION OF CITRON

The first step in the commercial curing process is to place the longitudinally cut halves or caps of unripe citron into tuns or hogs-

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heads filled with either sea water or a 5- to 8-percent common salt solution. At outdoor temperatures the strength of the brine is renewed after about 15 days because of dilution. After 40 to 50 days, when the caps are transparent and cured, the brine is made up to approximately 10 to 12 percent of salt for shipping and long storage. Citron thus treated is imported into the United States from Italy, Greece, and Corsica.

The experimental fermentation took place at room temperature in large crocks containing a 5-percent solution of sodium chloride
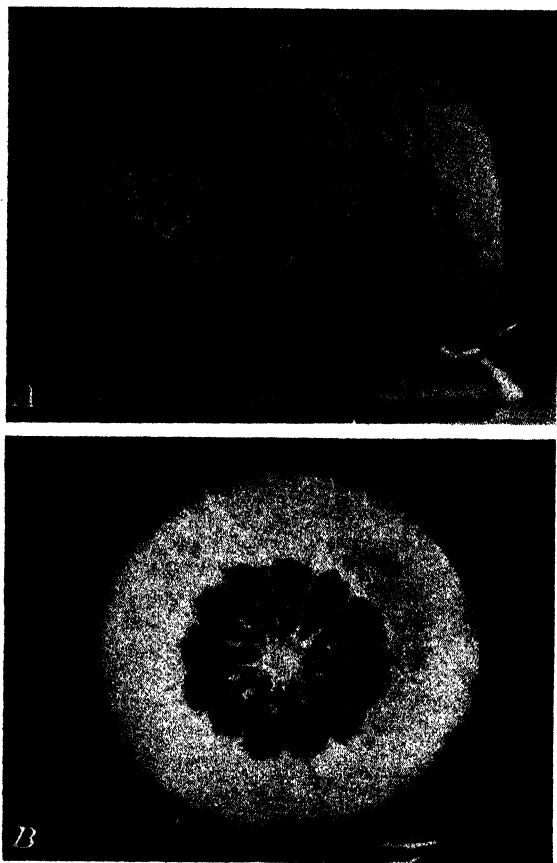
FIGURE 1.—*A*, A fresh green citron showing the prominent oil sacs in the epicarp; *B*, cross-section of a citron showing the fleshy mesocarp used for preserving.

covered with a layer of paraffin. The strength of the brine was renewed after 15 days, and after 35 days the citron caps were translucent, clear green, and showed no opaque spots. The fermentation was entirely normal. The characteristic yeasts and bacteria isolated by Hollande and Chadefaux (8) were found in large numbers in the brine. Scum yeasts were also plentiful after 10 to 15 days, but were kept under control by the paraffin layer at the surface. The acidity of the brine increased steadily from 0 at the beginning of the experiment to 7 g of acid calculated as acetic per liter after 14 days. Lactic acid was

present as shown by qualitative tests. After 14 days the acidity remained nearly constant, although a decline to 6 g per liter was observed in some crocks. This was probably due to the destruction of acid by scum yeasts. In general, the rise in acidity coincided well with increased counts of yeasts and bacteria. The latter increased steadily for 20 days. The yeasts seemed to reach a maximum at about 10 to 15 days; after this period counting was difficult because of the presence of scum yeasts and debris. Both direct counts of total cells and plate counts of viable cells were made. The direct counts far outnumbered the agar plate counts. Detailed cultural studies of the micro-organisms in the fermenting brine fully confirmed the findings of Hollande and Chadefaux (8) and of Chadefaux (3) as to the character of the micro-organisms. These were designated *Saccharomyces citri medicae* and *Bacillus citri medicae*. Thus it appears that the fermentation of Puerto Rico citron is very similar to that of Corsican citron, and is due to the same organisms. The fermented citron soon softens excessively and loses its characteristic flavor and color unless the concentration of the brine is raised to 10 or 12 percent. At cool temperatures well-brined citron will keep for 2 years or more. The use of sulphur dioxide (SO_2) or sodium sulphite (Na_2SO_3) in sufficient concentrations to give 200 to 500 parts per million of SO_2 is also useful for preserving the fermented citron in brine. Even citron in 5-percent brine may be thus perfectly preserved. The SO_2 is readily removed along with the salt in the freshening process.

PRESERVING THE FERMENTED CITRON

The final manufacturing process consists of first desalting the citron halves by boiling them in twice their weight of water until the flesh is tender. The citrons then are soaked in running water for several hours. The salt content is reduced to 1 to 2 percent. The peel is translucent, semisoft, and free from opaque spots. The pulpy centers are removed by hand and discarded, and the citrons are then preserved by gradually building up, over a period of several days, the sugar concentration of the flesh by the use of hot sirups. The first sirup contains 15 to 20 percent of sugar, the final one about 75 percent. The sugars used are usually mixtures of cane or invert sugars and glucose. After the sirup has been drained off the citron is dried in a hot-air kiln until the moisture content is reduced to 18 to 25 percent. It is then ready for packing or dicing for the retail trade. There are many variations of the foregoing method but the resulting products are very similar. Candied or glacé citron is prepared by simply dipping the peel in boiling supersaturated sucrose sirup. General data on brining and candying of citron are given by McCulloch (11), Cruess and Glickson (5), and Chace (2). The preserving sirups used in this study contained 65 percent of sucrose and 35 percent of glucose sirup, and the preserved citron contained 70 to 74 percent total sugars (sucrose and glucose). The final moisture content of the preserved peel was between 17.0 and 19.5 percent. The preserving process took 10 days. The peel was dried for 12 hours at 120° F. in a current of air. Sample lots of peel were also preserved by using pure sucrose and different percentages of sucrose and glucose sirup. Some of the lots were also dried to various moisture contents which ranged from

17 to 25 percent. The pure sucrose gave a peel that was too firm in texture and of poor keeping quality because of sugar crystallization. The use of glucose sirup in the preserving process greatly improved the quality of the finished peel. There was no mold or fermentation on peel having a moisture content of 17.0 to 18.5 percent, even after long storage.

CHEMICAL COMPOSITION OF FRESH GREEN AND PRESERVED CITRON

The methods of the Association of Official Agricultural Chemists (1) were followed in making the analyses. Copper was determined by the method of Lindow, Elvehjem, and Peterson (10), iron according to Stugart (18), and iodine by Morse's modification of the Von Fellenberg method (12). The results of the analyses are shown in table 1, and because of the scarcity of published data, several partial analyses found in the literature are also included. The pulpy portion of the fruit, including the seeds, was removed from all samples before analysis; the results therefore represent the edible portion only.

The moisture content of citron is somewhat higher than that of other citrus fruits, and the ash, protein, and ether extract are about the same. In spite of the firm mesocarp, the citron is very low in crude fiber and must consist largely of digestible carbohydrates rather than lignin. There is not as much difference between the unripe fresh fruit and the preserved fruit as might be supposed. The total sugars as invert in the fresh fruit are rather low, only 1.55 percent, though the total carbohydrates (by difference) are fairly high, that is, 9.41 percent.

TABLE 1.—*Chemical composition of fresh green and preserved citron*

| Determination | Data from this investigation | | | Data from Sherman (15), preserved fruit | Data from König (9 Bd. 1, p. 912), preserved fruit | Data from Hartel and Kirchner(7), preserved fruit |
	Fresh unripe fruit	Preserved fruit	'Commercially preserved product			
Moisture _____percent..	88.56	18.81	17.00	19.0	2.91	_____
Ash _____do____	.44	.48	.51	.9	_____	_____
Protein (N×6.25) _____do____	.15	.16	.18	1.5	_____	_____
Ether extract _____do____	.32	.33	.38	1.5	_____	_____
Crude fiber _____do____	1.09	1.37	1.89	_____	3.69	_____
Total sugars (invert) ____do____	1.55	43.05	45.81	_____	30.90	_____
Glucose _____do____	_____	28.14	32.36	_____	29.87	11.5–30.8
Sucrose _____do____	_____	14.91	13.45	_____	1.01	14.3–50.0
Total carbohydrates (except fiber) _____percent____	9.41	78.85	72.60	78.1	_____	_____
Calcium oxide _____do____	.09	.06	.12	.17	_____	_____
Phosphorus pentoxide ____do____	.046	.038	.05	.151	_____	_____
Potassium oxide _____do____	.114	.021	_____	.51	_____	_____
Ferrous oxide _____p. p. m..	9	4.1	16	_____	_____	_____
Cupric oxide _____do____	1	1.7	6	_____	_____	_____
Iodine _____p. p. b..	3	(1)	(1)	_____	_____	_____
Alkalinity of ash [2] _____	6.9	8.3	7.0	9.8	_____	_____
Vitamin A [3] _____cc units..	0.8–1.0	(4)	_____	_____	_____	_____
Vitamin C [5] _____do____	6	(1)	_____	_____	_____	_____

[1] Trace.
[2] Cubic centimeter of normal HCl required to neutralize the ash from 100 g.
[3] International units per gram; 1 Sherman unit of vitamin A=1.4 international units.
[4] Less than 1.
[5] 1 international unit of vitamin C=0.05 mg l-ascorbic acid. Protective level for guinea pigs was determined to be 0.5 mg l-ascorbic acid.

A partial mineral analysis is given for both the fresh and preserved citron. As in other citrus fruits, the ash is definitely alkaline and there are appreciable amounts of calcium, phosphorus, and copper. The iron and iodine content is low. The increased amount of iron and copper in some of the preserved samples is probably due to contamination by metals during the manufacturing process. The presence of copper salts in minute quantities causes a noticeable greening of the epicarp. The presence of vitamins A and C is noted in table 1 for the sake of completeness. The vitamin studies are reported below.

VITAMIN C IN FRESH GREEN CITRON AND IN FERMENTED PEEL

The assay for vitamin C was conducted by the method of Sherman, LaMer, and Campbell (*17*), 300-g guinea pigs being fed for 90 days. The vitamin C-free ration kept before the animals at all times consisted of baked skim milk 30 parts, butter fat 10 parts, bran 29.5 parts, rolled oats 29.5 parts, sodium chloride and cod-liver oil, each 1 part. The animals were housed in individual wire cages and were fed at five levels of green citron and three levels of cured, fermented citron. Since guinea pigs dislike sweet foods, they were first fed the desalted fermented citron to deteimine whether any vitamin C remained in the cured fruit. Had some been found, it was intended to next assay the preserved citron. However, inasmuch as only a trace of vitamin C was found in the cured fermented product before the addition of sugar, there was no use in assaying the finished product, i. e., the preserved or candied citron. The desalting was accomplished by boiling the brined peel in water for 1 hour. The ratio of peel to water by weight was approximately 2 to 1.

Sectors of green citron, exclusive of pulp and seeds, were fed in these tests. The animals ate both the green and the cured citron without forced feeding. Three guinea pigs were fed at each level. Average weight gains and scurvy scores at autopsy are shown in figure 2.

The graphs in figure 2 show clearly that the green raw citron is an excellent source of vitamin C, the protective dose being very slightly more than 2 g, or approximately 5 to 6 international units per gram based on an estimated protective level of 0.5 mg of ascorbic acid. At the 2-g feeding level the average weight gain per guinea pig was 250 g with only traces of scurvy observed at autopsy. At all higher levels, protection was complete and very large weight gains were recorded for all the animals. The 10-g level of the brined, desalted citron is not shown in figure 2 since the three guinea pigs died within 35 days with an average scurvy score of 9.

The brined, fermented citron showed but a trace of vitamin C, as 15 g gave very little more protection than the basal ration (negative control). That a slight amount of vitamin C was present is indicated by the fact that the guinea pigs receiving 15 g lived 10 to 15 days longer than those receiving only the basal ration. It is safe to conclude from this experiment that the preserved or candied citron which is made from the desalted, brined fruit contains little or no vitamin C. The causes of the loss of vitamin C during fermentation in a 5-percent NaCl brine are not clear. There is much carbon dioxide given off during the fermentation, and since the citrons are under the brine, there is little chance for oxidation. Upon removal from the brine,

the caps were placed directly in boiling water and kept there until they were well desalted. The desalted citron was sliced and fed to the animals as soon as it was cool.
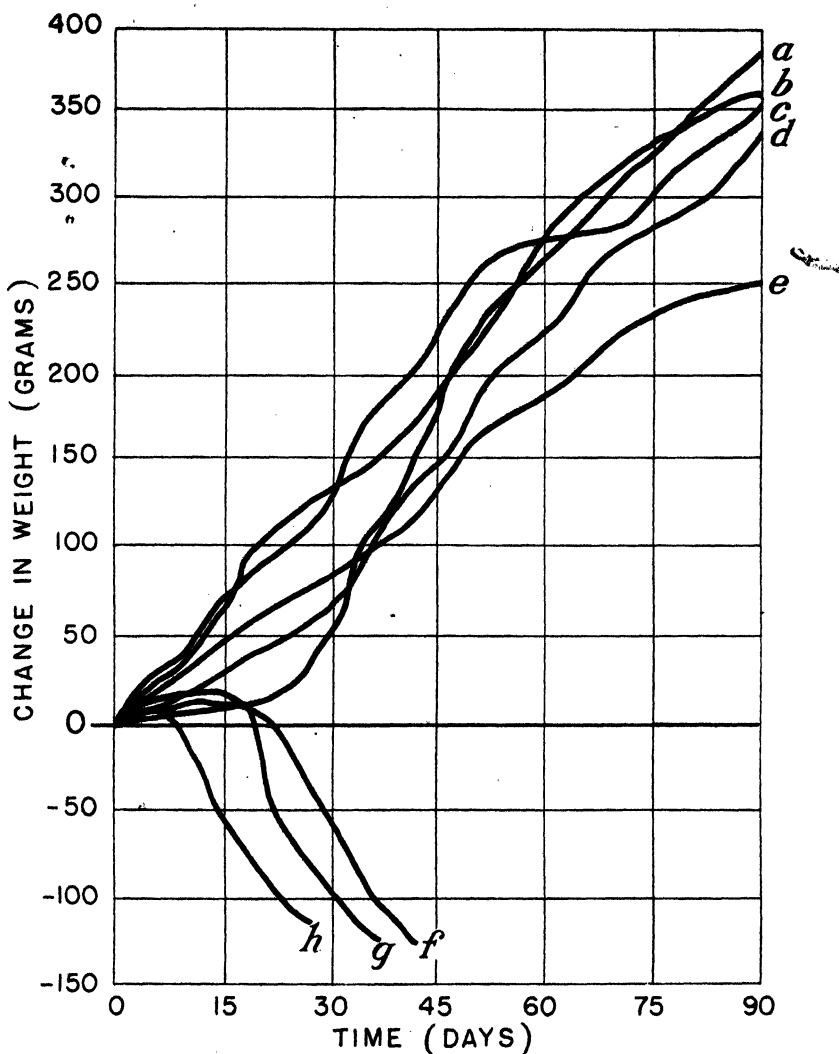
FIGURE 2.—Results of feeding guinea pigs green and brined citron as the sole source of vitamin C: *a*, 12 g green citron fed daily, scurvy score, 0; *b*, 4 g green citron fed daily, scurvy score, 0; *c*, 15 g green citron fed daily, scurvy score, 0; *d*, 6 g green citron fed daily, scurvy score, 0; *e*, 2 g green citron fed daily, scurvy score, trace; *f*, 15 g desalted fermented citron fed daily, scurvy score, 15; *g*, 12 g desalted fermented citron fed daily, scurvy score, 14; *h*, negative control, scurvy score, 19.

The results here reported compare favorably with those of Thurman and Vahlteich (*19*), who found that cucumber pickles manufactured from brined stock gave no measurable protection from scurvy at the 15-g level. Similarly, Eddy and his associates (*6*) showed that cabbage lost about 95 percent of its vitamin C when thoroughly cooked

in an open kettle. Clow, Marlatt, et al. (4) and also Parsons and Horn (14) found that cabbage suffered marked losses in vitamin C when it was fermented into sauerkraut.
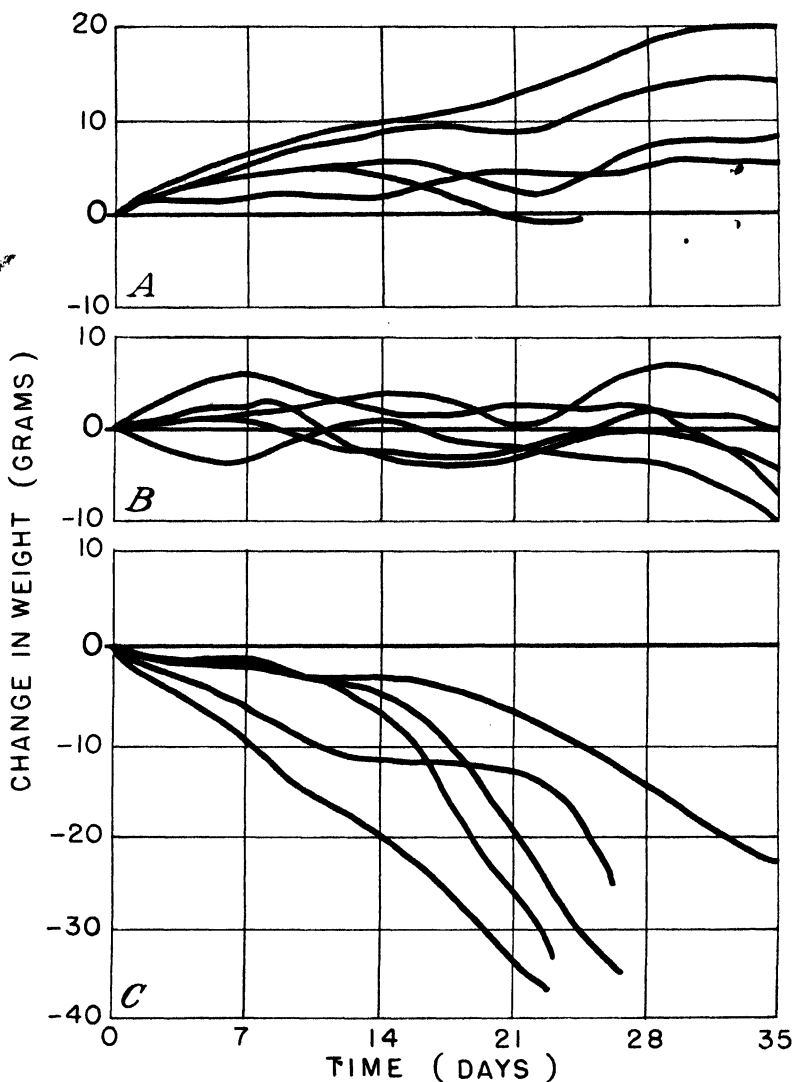
FIGURE 3.—Results of feeding fresh green and preserved citron as the sole source of vitamin A to albino rats previously depleted of this vitamin: *A*, 1 g fresh green citron; *B*, 1 g preserved citron; *C*, negative control receiving vitamin A-free basal ration only.

VITAMIN A IN FRESH GREEN AND PRESERVED CITRON

For determining vitamin A the Sherman and Burtis (16) method was used. Young albino rats weighing from 35 to 50 g were fed a vitamin A-free basal diet consisting of cornstarch 67 parts, vitamin-free casein 18 parts, irradiated yeast 10 parts, sodium chloride 1 part,

and Osborne and Mendel's (*13*) salt mixture 4 parts. Normally, the animals are depleted of vitamin A after 4 to 5 weeks on this ration, but in this experiment a somewhat longer period was required before xerophthalmia was observed. Five rats were used at the 1- and 0.25-g levels for both the green fresh citrons and the preserved peel (candied citron), and eight were used for negative controls. Three of the latter died within 2 weeks, and the data for these are not included in the graph for negative controls (fig. 3, C). The sample of preserved peel that was fed contained 19 percent moisture.

The data for the three groups of rats are presented in figure 3. Only the graphs showing weight gains of the rats on the 1-g level are given because of the incomplete protection from xerophthalmia and infections of the sinuses and middle ear of the rats fed 0.25 g. There is some indication that the green fresh citron was somewhat richer in vitamin A than the preserved citron. The latter, however, contains over 50 percent of sugar. In no instance did the citrons give weight gains of 3 g a week, the equivalent of 1 Sherman unit. The vitamin A content of the green fresh citron is estimated roughly at 0.8 to 1.0 international unit, and the preserved citron at slightly less.

SUMMARY

The citron is characterized by high moisture and medium sugar, ash, and fat content. The fiber content is low. Ash analysis shows moderate amounts of calcium, potash, phosphorus, and copper. The iodine content is low.

The fresh unripe citron is very rich in vitamin C, about 2 g being the protective amount for guinea pigs. This corresponds to 6 international units per gram. There is very little retention of vitamin C in the brine-fermented citron and probably none in the candied peel.

The vitamin A content of the green fresh citron is estimated at 0.8 to 1 international unit and that of the preserved (candied) peel at slightly less.

The findings of Hollande and Chadefaux in regard to the micro-organisms responsible for the characteristic brine-fermentation of Corsican citron are corroborated for Puerto Rican citron. A yeast, *Saccharomyces citri medicae*, and *Bacillus citri medicae*, were found in large numbers in the fermenting brined citron. The acid production in 15 days, during fermentation, averaged 6 to 7 g per liter (acetic). The flavor and aroma of citron are largely developed during the fermentation.

In preserving citron the use of 35 percent of glucose with the sucrose yields a peel of excellent flavor, texture, and color. A moisture content of preserved (candied) citron peel of 17 to 19 percent is sufficiently low to prevent yeast fermentation or mold growth.

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EFFECT OF FERTILIZER ON QUALITY AND CHEMICAL COMPOSITION OF CANNING PEAS[1]

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INTRODUCTION

Many investigators have studied the chemical composition of fresh peas under various conditions (*1, 5, 12, 13*),[3] and some have studied the composition of canned peas (*26*). Differences in the quality of the peas due to differences in composition have generally only been inferred. Few investigators have considered the effect of plant nutrients on the chemical composition of peas (*11, 23*), or determined the ultimate effect of plant nutrients or chemical composition on the quality of the canned product as determined by experienced judges. (*15, 23*).

In the present investigation field trials were conducted to determine the influence of fertilizers on the yield and quality of canned peas. Chemical analyses were also made on the fresh whole peas and on pea skins to determine, if possible, the manner in which the changes in quality were brought about.

FIELD METHODS

Two series of plots were laid out in 1931 in the two most important pea-canning areas of Wisconsin. One series was located at Dorchester on Colby silt loam, an old glacial soil derived largely from granitic material, which had a pH value of 5.5 to 6.0. The other series was located at Ripon on Miami silt loam, a soil derived from limestone. The pH value of this soil was 6.6 to 6.8.

Each series of plots included the eight treatments indicated in table 1. The fertilizers were applied broadcast at the rate of 300 pounds per acre and were disked in before planting. The potassium in the fertilizers was derived from muriate of potash, the phosphorus from treble superphosphate, and the nitrogen from ammonium sulphate (70 percent) and from nitrate of soda (30 percent). The Perfection variety was planted on the Colby plots at the rate of 4½ bushels per acre, and the Advancer variety on the Miami plots at the rate of 4 bushels per acre. All the seed was inoculated.

The Miami plots were one-tenth of an acre in size, and the Colby plots were one-fifteenth of an acre. All treatments were duplicated. The Miami plots were seeded on April 22 and harvested on July 3. Hot weather and the danger of increased aphid infestation made it necessary to harvest these plots before the peas had reached the normal canning stage. The Colby plots were seeded May 4. One set of the duplicate plots was harvested on July 13 and the other set on July 15.

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The peas from each plot were vined, washed, and graded separately, and each grade (size) from each plot was blanched, canned, and processed separately.[4] The time when each plot was cut, vined, washed, and blanched was recorded. An effort was made to reduce the time between vining and washing to a minimum and to keep this time interval uniform for all plots. Since the viners were located at some distance from the canneries, however, from 1¼ to 3¼ hours elapsed between the time the peas were vined and the time they were washed.

PLOT YIELDS AND QUALITY OF THE CANNED PRODUCT

After the canned peas had been stored for several months, representative cans of each size, from no. 1 through no. 5, were scored by experienced judges. The acre yields and average score of the peas for the various treatments are given in table 1.

TABLE 1.—*Effects of different fertilizer treatments on yield and quality of two varieties of canning peas*

Fertilizer [1] mixture	Perfection variety on Colby plots		Advancer variety on Miami plots	
	Yield per acre	Score [2]	Yield per acre	Score [2]
	Pounds		*Pounds*	
Blank	1,351	84.1	675	80.0
0-16-0	1,294	80.5	678	87.6
0-16-8	1,182	84.2	679	84.0
2-16-4	1,535	86.1	685	93.0
2-16-8	1,622	86.9	712	91.0
2-16-16	1,545	86.2	784	93.0
4-16-16	1,588	84.2	806	88.2
4-16-8	1,518	84.7	636	84.5

[1] Proportions of nitrogen, phosphorus, and potash .
[2] Average of all sizes.

Peas from some of the Colby plots harvested on July 13 were lost, and only the results of the second harvest are given. Rather high temperatures necessitated harvesting the Colby plots somewhat before the peas had reached the normal canning stage; consequently, the yields are slightly below normal. Temperatures above normal and injury from aphids so reduced the yields on the Miami plots that the duplicate plots had to be combined in order to get sufficient material to process each size of pea separately. It had been planned to make two separate harvests on both the Colby and the Miami plots so that if a certain treatment tended to hasten maturity, the peas would be cut at the proper stage of maturity at the early harvest, and if some other treatment tended to delay maturity, the peas would be cut at the proper stage of maturity at the later harvest. Under the conditions prevailing in this investigation these plans could not be carried out.

On the Colby silt loam, those plots that received complete fertilizers produced somewhat higher yields than the unfertilized plots, while the plots that received only phosphorus or phosphorus and potash without nitrogen yielded less than the unfertilized plot. The differences in yield between fertilized and unfertilized plots on the

Miami silt loam were not very great because of abnormally high temperatures, aphid infestation, and early harvesting. The highest yields, however, were obtained by the use of complete fertilizers, excepting the 4–16–8 treatment.

The National Canners Association, in their score card for quality of canned peas, give 35 points for tenderness, 25 for flavor, 15 for clearness of liquor, 15 for freedom from broken peas and other defects, and 10 for uniformity in size and color. The last three items are controlled almost entirely by factory practices. Any effect of fertilizer on the quality of canned peas must, therefore, be sought in variations in tenderness and flavor. Canning practices may influence to some extent the flavor and possibly the tenderness of peas, but these factors depend largely on the stage of maturity of the peas and on the variations in chemical composition and cellular structure that accompany variations in maturity.

There were no pronounced differences in the quality of the peas from the Colby plots, but the peas that scored highest were from the plots that received complete fertilizers containing 2 percent of nitrogen, 16 percent of phosphoric acid, and potash varying from 4 to 16 percent. When the nitrogen in the fertilizer was increased to 4 percent, as in the 4–16–8 and 4–16–16 treatments, or left out entirely, as in the 0–16–0 and 0–16–8 treatments, the quality of the peas produced was no better than that of the peas from the unfertilized plot. The 0–16–0 treatment produced the lowest quality peas.

In the Miami silt loam, all of the plots that received fertilizer produced peas of distinctly better quality than the unfertilized plot. As in the Colby silt loam, those plots that received fertilizers containing 2 percent of nitrogen, 16 percent of phosphoric acid, and potash varying from 4 to 16 percent (2–16–4, 2–16–8, and 2–16–16 treatments) produced the highest scoring peas. Increasing the nitrogen to 4 percent, as in the 4–16–8 and 4–16–16 treatments, or leaving the nitrogen out entirely, as in the 0–16–0 and 0–16–8 treatments, resulted in the production of peas that were higher in quality than peas from the unfertilized plot, but lower in quality than the high-scoring peas from the three previously mentioned plots.

The relative differences in quality between treatments, as shown by the average score of all sizes, was in general also exhibited separately by each of the five sizes. For example, the peas from the 2–16–16 plot (Miami silt loam) scored 90, 95, 95, 95, and 90 for the sizes 1 through 5, respectively, giving an average score of 93. The 4–16–16 scored 90, 90, 90, 90, and 81 for the same sizes, giving an average score of 88.2. The peas from the unfertilized plot were the most variable, scoring 86, 76, 81, 81, and 76 for the sizes from 1 through 5, respectively, giving an average score of 80.

The liquor was reported as clear for the cans of peas from all of the plots on the Miami soil. The flavor was reported as being flat for the no. 1 peas from the unfertilized, 0–16–8, and 4–16–8 plots; for all of the other sizes the flavor of the peas from all of the plots was reported as generally good. Tough peas were reported (Miami soil) for all sizes of peas from the unfertilized and the 0–16–8 plots, and for several sizes from the plots receiving the 0–16–0, 4–16–8, and 4–16–16 treatments. Only the no. 1 peas from the 2–16–16 plot were pronounced tough, and the no. 3 peas from the 2–16–8 plot were reported as slightly so. The tenderness of the peas of all sizes from

the 2-16-4 plot was good. From the Miami plots one can thus conclude that the effect of fertilizers on quality of canned peas is due largely to changes in the tenderness of the peas, but the judges' reports indicate that flavor may also be influenced by fertilizers. The peas from the Colby plots did not exhibit such decided differences in tenderness.

The effect of fertilizers, especially of well-balanced complete mixtures, was to improve the culinary qualities of peas grown on the soil types under investigation and to increase the yield of peas. From the results reported some general conclusions may be drawn as to the approximate composition of a fertilizer that will improve the quality as well as increase the yield of the pea crop. When applied at the rate of 300 pounds per acre, such a fertilizer (for Wisconsin conditions on the Colby and Miami silt loams) should contain about 2 percent of nitrogen, 16 percent of phosphoric acid, and from 4 to 16 percent of potash.

CHEMICAL COMPOSITION OF PEA SKINS

Peas for the seed-coat analyses were withdrawn from the plot lots after they had gone through the washer and graders. The seed coats were removed by hand as soon as possible. About 5 g of the fresh skins was preserved for total nitrogen determination, and about 50 g for calcium, potassium, and phosphorus. Accepted methods of quantitative analysis were followed in determining the amounts of mineral elements.

TOTAL NITROGEN

The standard Kjeldahl procedure was followed in determining the total nitrogen content of the pea skins. The results are given in table 2.

TABLE 2.—*Effect of different fertilizer treatments on the total nitrogen content (dry basis) of skins from no. 2, no. 3, and no. 4 peas grown on the Colby plots*

Fertilizer mixture	Nitrogen in skins of—			Fertilizer mixture	Nitrogen in skins of—		
	No. 2 peas	No. 3 peas	No. 4 peas		No. 2 peas	No. 3 peas	No. 4 peas
	Percent	Percent	Percent		Percent	Percent	Percent
Blank	4.79	3.70	3.39	2-16-16	4.04	3.82	3.51
0-16-0	4.18	3.86	3.47	4-16-8	4.37	3.82	3.38
0-16-8	4.46	4.14	3.55	4-16-16	4.13	3.64	3.38
2-16-8	4.40	3.62	3.43				

[1] See footnote 1, table 1.

Marked differences are found in the nitrogen content of pea skins of different sizes. All fertilizers apparently lowered the nitrogen content of the no. 2 peas, but with the larger sized peas this effect is not consistent. The general trend, irrespective of treatment, shows a consistent decrease in the nitrogen of the seed coat of peas with advancing maturity, assuming that the larger peas are more mature. This fact indicates that the total nitrogen content of pea skins is primarily a function of maturity and not one of nitrogen added in the form of commercial fertilizer.

If the assumption is correct that larger peas are more mature (*19, 23*), then the smaller percentage of nitrogen in the larger peas may be explained by the fact, established by Bisson and Jones (*1*), that total nitrogen increases less rapidly than carbohydrates and other nonnitrogenous constituents. Thus it appears that the total nitrogen in the skins is influenced by the maturity factor in a way similar to the whole pea.

TABLE 3.—*Effect of different fertilizer treatments on the calcium, phosphorus, and potassium content of skins from peas grown on the Colby plots*

Fertilizer mixture	Pea size no.	Calcium on—		Phosphorus on—		Potassium on—	
		Ash basis	Dry matter basis	Ash basis	Dry matter basis	Ash basis	Dry matter basis
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Blank	2	3.79	0.151	11.29	0.450	32.60	1.30
	3	4.39	.178	10.17	.414	38.06	1.55
	4	5.21	.208	8.33	.333	33.24	1.33
0-16-0	2	3.40	.147	12.48	.541	32.44	1.41
	3	3.96	.167	11.25	.475	32.56	1.38
	4	4.39	.190	9.93	.480	32.47	1.41
0-16-8	2						
	3	3.45	.155	11.03	.496	30.65	1.38
	4	4.06	.176	11.02	.478	31.88	1.38
2-16-8	2	3.46	.145	12.08	.507	32.47	1.36
	3	4.07	.173	10.36	.441	33.58	1.43
	4	5.20	.217	8.97	.375	31.42	1.32
2-16-16	2	3.52	.165	13.38	.627	31.77	1.49
	3	3.90	.170	11.47	.496	33.33	1.44
	4	4.73	.200	9.23	.390	30.70	1.30
4-16-8	2						
	3	4.12	.169	11.19	.468	28.07	1.15
	4	4.78	.204	10.60	.452	29.21	1.25
4-16-16	2	3.70	.157	13.55	.574	29.49	1.34
	3	3.97	.172	11.38	.497	31.92	1.38
	4	4.87	205	8.83	.393	32.13	1.36

[1] See footnote 1, table 1.

PHOSPHORUS

Table 3 gives the percentage of phosphorus, potassium, and calcium in the pea skins. The phosphorus content of the various fertilizer mixtures was uniformly 16 percent. In practically every case, the phosphorus in pea skins decreases regularly with increase in size of pea, following in general the nitrogen trend. Phosphorus accumulates in relatively large amounts in the ovules in the earlier stages of growth and this appears also to be true of the seed coats. Rather striking increases in phosphorus in the seed coats are noted. These increases are consistent and particularly marked in the no. 2 and no. 4 peas, amounting to 25 and 28 percent, respectively (average of all fertilizer treatments).

POTASSIUM

No such definite relationships are noted in potassium as in nitrogen and phosphorus. Nor did the inclusion of various amounts of potassium in the fertilizer exert any consistent influence on the potassium content of the seed coats. This is particularly noticeable if such treatments as 0-16-0 and 0-16-8 or 2-16-8 and 2-16-16 are compared. The skins of no. 3 peas from the unfertilized plot contained a larger percentage of potassium than the skins of no. 3 peas from any of the fertilized plots. The peas on the unfertilized plot apparently were able to obtain as much potassium as those on plots receiving potash

fertilizers. It is possible that the potassium content of the rather inert skin tissues is quite constant and does not vary greatly even though more potassium may be present in the vegetative part of the plant (*23*).

The potassium comprised from 28 to 38 percent of the ash of pea skins. A similar high concentration of potassium in pea skins has been reported by others (*23*). From 8.3 to 13.5 percent of the ash of pea skins was phosphorus, and from 3.4 to 5.2 percent was calcium. It is not known what significance, if any, this high percentage of potassium in the ash of pea skins has.

CALCIUM

It will be remembered that tenderness is given more weight than any other single factor in scoring canned peas for quality. Tough seed coats are associated with a lack of tenderness in peas, although it must not be overlooked that the character of the pulp may also influence tenderness. This is true whether mechanical means (*23*) are used to determine tenderness or whether it is determined by experienced judges.

The results of numerous investigations indicate a relationship between calcium and potassium in plant tissue. Fonder (*7*) reports that in alfalfa a high potassium content is usually accompanied by a low calcium content, and, inversely, a low potassium content is accompanied by a high calcium content. A similar relationship has been reported between calcium and potassium in peas (*23*, *25*). Sayre, Willaman, and Kertesz (*23*) found that calcium in pea ovules decreases as the potassium increases, and that a higher calcium content is always associated with tougher peas.

In the present investigation, a relationship was observed between the calcium content of the pea skins and potash in the fertilizers. A comparison of the seed coats of peas from the 0–16–0 and the 0–16–8 plots shows that the addition of potash reduced the calcium in the seed coats of the no. 3 and no. 4 peas. A comparison of the seed coats of peas from the 2–16–8 and 2–16–16 treatments gave similar results, except in the case of no. 2 peas. The differences between the effect of the 4–16–8 and the 4–16–16 treatments are negligible. In general, potash in the fertilizer served to diminish the concentration of calcium in the seed coats of peas, a finding which is in harmony with those reported by others (*23*, *25*).

It is apparent that maturation also influences calcium concentration in the seed coat. Without exception, a definite increase in the calcium of the seed coats is noted with an increase in size of peas. On the plots receiving potash the average increase in calcium of the seed coats of no. 3 over no. 2 peas is about 10 percent and of no. 4 over no. 3 about 20 percent. A similar condition is found in the unfertilized plot, but the magnitude of the increase is even greater in the no. 3 as compared with the no. 2 peas. Since calcium migration to the skin is an old-age phenomenon, it is very probably associated with the deposition of old-age organic constituents, such as hemicelluloses, lignins, or pectates. In the case of whole peas the calcium content appears to be fairly constant irrespective of the stage of maturity (*5*, *23*, *26*).

It is a striking fact that all of the fertilizer treatments reduced the calcium concentration in the seed coats, whether phosphorus alone,

phosphorus with potash, or all three elements were provided. It is possible that larger yield and more active metabolism diminished the proportion of calcium migrating to the skin and also the ratio of skin to total seed. It is to be noted, however, in comparing the various fertilizer treatments, that there is no definite relationship between the relative percentages of calcium found in the pea skins and the quality of the canned product. For example, the peas from the 2–16–8 treatment received the highest score, but the pea skins from this plot had a higher calcium content than the pea skins from most of the other fertilized plots. Also, the lowest concentration of calcium was in the seed coats of peas from the plot receiving the 0–16–8 treatment. This treatment did not give the highest scoring peas.

Calcium probably occurs in plant tissues not in the inorganic state but combined or associated with organic constituents such as lignins, pectates, and hemicelluloses. These organic constituents may have a direct influence on the quality of peas by affecting the cellular structure and thus the tenderness and flavor of the peas. Calcium associated with these organic constituents may thus be but an indirect indication of quality in peas.

CHEMICAL COMPOSITION OF WHOLE PEAS

The starch content of whole peas may vary from less than 6 to over 37 percent and the total sugars from above 35 to less than 4 percent (*1, 5, 23*). The relative amounts of these two constituents in peas have always been considered important in affecting quality.

Since stage of maturity is of prime importance in determining sugar and starch content, and thus the flavor and tenderness of peas, many workers have investigated the organic constituents of peas at various stages of growth. Boswell (*2, 3, 4, 5*) states that the growth and maturity process is characterized by a rapid decrease in percentage of sugar and soluble nitrogenous substances, and by an increase in starch, total and acid-hydrolyzable substances, and insoluble nitrogen compounds. Sayre and his coworkers (*23*) report a decrease in sugar and an increase in total organic matter and protein as the pea ovule matures. Bisson and Jones (*1*), in an investigation covering 5 weeks' development, during which time 14 samplings were made, found that peas contained a maximum of 33.5 percent of sucrose, but that before the peas were at the proper stage for harvesting the sucrose content had dropped to 20.5 percent. They found that the total sugar in peas per pod continued to increase slowly while the percentage was decreasing until the proper stage for harvesting was reached, when the total sugar per pod and the percentage both decreased. The ratio of starch to sugar has been used by various investigators (*2, 5, 19*) to correlate what was considered the most important chemical changes in the development of the pea with maturity and quality.

Sufficient evidence is thus at hand to show that the quality of peas changes with stage of maturity and that this change in quality is accompanied by a decrease in sugars and an increase in starch. However, it has not been determined whether nutrient elements supplied in fertilizers can affect the organic composition of the pea and thus the quality. Jodidi and Boswell (*11*), working with an abnormal soil and using heavy applications of nitrogen, phosphorus, and potash singly, concluded on the basis of 2 years' work that the application of

nitrogen to peas (116 pounds of nitrogen per acre) resulted in a higher percentage of sucrose and a distinctly lower starch content. The phosphorus treatment (160 pounds P_2O_5 per acre) was followed by no noticeable difference in percentage of carbohydrate constituents, ash, or of the ether extract. One hundred and forty-four pounds of potash per acre apparently had no effect on the sugar or starch content. Sayre's work (*22, 23*) shows quite conclusively that the mineral composition of peas and of pea skins is affected by the nutrients supplied and that high calcium in the seed coat is associated with a tougher and harder pea, which results in a lower quality canned product. Sayre found that the structure of the cells of the pea ovule was definitely affected by differences in the nutrients supplied. However, he concluded (from water-culture experiments) that the pea plant can vary its mineral content and the ratios among these minerals over a very wide range and yet not appreciably alter its ratio of carbohydrates and nitrogenous constituents. This may be an unsafe generalization, since desiccation and maturation of the pea ovule may be disturbed and delayed by the constant high plane of water in water cultures.

TABLE 4.—*Effect of different fertilizer treatments on the sugar and starch content starch-sugar ratios, and alcohol-soluble matter of no. 3 peas grown on the Colby and the Miami plots*

Fertilizer mixture [1]	Total dry matter soluble in 70-percent alcohol		Starch				Sugar				Starch-sugar ratio	
			Miami		Colby		Miami		Colby			
	Miami	Colby	Ratio to total dry matter	Total dry matter insoluble in 70-percent alcohol	Ratio to total dry matter	Total dry matter insoluble in 70-percent alcohol	Ratio to total dry matter	Total dry matter soluble in 70-percent alcohol	Ratio to total dry matter	Total dry matter soluble in 70-percent alcohol	Miami	Colby
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*		
Blank	40.0	49.2	12.98	21.6	9.65	19.0	20.17	50.3	29.36	59.5	0.643	0.328
0-16-0	43.8	48.5	11.06	19.7	9.66	18.7	22.41	51.1	28.59	58.8	.493	.337
0-16-8	43.6	52.4	11.62	20.6	7.72	16.2	22.18	50.8	26.81	51.1	.524	.288
2-16-8	39.9	41.9	11.91	19.8	11.50	19.7	21.41	53.6	31.80	75.7	.556	.361
2-16-16	41.7	48.2	11.62	19.9	9.92	19.2	24.05	57.5	29.09	60.2	.483	.341
4-16-8	36.4	50.7	13.05	20.5	8.68	17.6	18.40	50.5			.709	
4-16-16		46.9			9.91	18.6			20.88	44.1		.474

[1] See footnote 1, table 1.

Table 4 gives the results of sugar and starch analyses made on whole peas in connection with the fertilizer trials on quality. The peas for analysis were taken at the canning factory after they had gone through the washer and grader. They were preserved in 70 percent alcohol ($CaCO_3$ was also added to some), and the analyses were made according to official methods.[5] Sugar content represents sucrose, since reducing sugars were not present.

It would be expected that the peas having the highest sugar and the lowest starch content, and thus the lowest starch-sugar ratio, would give the highest quality peas when canned. There is, however, no consistent relationship between sugars and starch content and the

[5] Bertrand's method of reducing the copper in Fehling's solution, and Mohr's volumetric method of determining the cuprous oxide, were followed.

effect of fertilizer on quality. Moreover, it should be noted that while the Perfection peas from the Colby silt loam were considerably higher in sugar and lower in starch than the Advancer peas from the Miami soil, the latter produced a higher quality canned product.

It has already been mentioned that the peas were cut before they reached the normal canning stage. Most canners consider a pea crop at the proper canning stage when 24 to 32 percent of the crop consists of no. 1, no. 2, and no. 3 peas. The peas on the Miami plots averaged 57 percent and on the Colby plots 34 percent of no. 1, no. 2, and no. 3 peas. When peas are at this stage of maturity and are still high in sugars and low in starch other constituents may be more important in determining the quality of the canned product than the sugar and starch content.

The starch and sugar together comprised about 30 to 40 percent of the total dry matter of the peas. While these two constituents undoubtedly are important in determining quality, the importance of the various nitrogenous constituents and carbohydrates other than sucrose and starch has been stressed by numerous investigators. Boswell (5) suggested the use of an insoluble-soluble nitrogen ratio but did not use it because its effect on quality is not so well known. Attempts have been made in Germany (17), France (8, 19, 20, 21), and the United States (15) to detect adulterated peas by means of various ratios of organic constituents other than starch and sugars. While it is important that all these constituents should be considered in any analysis for quality, no one has yet been able to set any definite ratio of any constituents as a critical or deciding point between superior and inferior quality peas.

Numerous investigators (6, 10, 12, 13, 14, 15, 18) have reported on the changes in composition of peas after harvesting. Peas were kept for from several hours to a week at different temperatures and chemical changes noted. Fresh green peas which were kept for as long as a week at 0° C. showed no appreciable change in chemical composition. Sugar was lost rapidly if the peas were held at high temperatures, even if only for a few hours. Most writers have shown, or attempted to show, that with a decrease in sugar there is a concomitant increase in starch. If the data are examined carefully, the increase in starch and other polysaccharides is found never to equal the loss in sugar. Most of the sugar is lost in respiration, and the increase in percentage of starch, as Kertesz (14) has shown, is due to the fact that the same amount of starch after loss of sugar by respiration must give a higher percentage. In another investigation (15) where peas were held for varying periods of time, Kertesz and Green were not able to correlate observed changes in quality of the canned product with changes in organic constituents.

The loss of sugars may be the primary cause of deterioration in the quality of market peas, but this can hardly be the explanation of the deterioration in canned peas since sugar is added in considerable quantity to the brine used in canning. This addition of sugar does not make the flavor of low-grade peas equal to that of choice peas.

SUMMARY

The influence of various fertilizers on the yield and quality of canning peas was studied in the field in 1931 on the Colby and Miami silt loams of Wisconsin. Properly balanced fertilizers increased the

yield of peas and also improved the quality of the canned peas, as determined by experienced judges.

Calcium, potassium, phosphorus, and total nitrogen determinations were made on the seed coats of no. 2, no. 3, and no. 4 peas from the Colby fertilizer plots. All of the fertilizers decreased the calcium and increased the phosphorus content of the seed coats of the three sizes of peas studied. The variations in calcium content of the seed coats bore no definite relation to quality of the canned product. The nitrogen and potassium content of the pea skins was not consistently affected by the fertilizer treatments. The skins of larger peas consistently contained more calcium and less phosphorus and total nitrogen than those of the smaller peas.

Sugar and starch analyses of no. 3 peas from the various fertilizer plots of both the Colby and Miami soils were made. Variations in quality of the canned peas could not be explained by differences in the sugar and starch content of the fresh peas. The peas on all plots were harvested at a relatively early stage of maturity, with the result that the peas were relatively high in sugar and low in starch. It is probable that variations in quality of canned peas harvested at such relatively early stages of maturity are determined largely by variations in organic constituents other than sugar and starch.

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DEVELOPMENTAL MORPHOLOGY OF THE CARYOPSIS IN MAIZE [1]

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INTRODUCTION

In recent years the maize plant has been the subject of extensive genetical, cytological, and physiological investigations. Many of these investigations have been concerned primarily with the kernel or caryopsis, while others have required a prerequisite knowledge of the developmental morphology of the plant, especially in the early stages. The object of the present study was to trace the developmental morphology of the kernel in an effort to determine not only the manner of development but also the order and rate of development of the various parts. Such a study of the normal development of the caryopsis in ordinary varieties would serve as a starting point for investigations of the various kernel types in numerous other varieties and abnormal genetical strains of maize. It was hoped that the investigation might clarify certain questions concerning the comparative morphology of maize and related grasses and also provide a more adequate basis for researches on maize in allied fields of investigation.

REVIEW OF LITERATURE

The developmental morphology of the caryopsis in maize (Zea mays L.) has been studied by numerous investigators, beginning with the classic researches of the early morphologists published during the middle of the last century. References to Zea are to be found in the important works of Schleiden (37),[3] Hofmeister (21), Schacht (36), and Van Tieghem (46). As early as 1872 the highly specialized nature of the maize embryo was fully recognized by Van Tieghem, who considered it a type distinct from that of other grasses, a distinction which has since come to be generally recognized. Prior to 1900 the general morphology of the caryopsis had been described and compared with that of related forms by True (47), Kennedy (23), and others. The essential features of double fertilization in maize were clearly

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outlined by Guignard (*16*) in 1901, 3 years after Nawaschin (*31*) discovered the process in *Lilium*. More recently Miller (*30*), Weatherwax (*51*), and Rhoades [4] have given accounts of fertilization in maize.

The early stages in embryogeny and endosperm development and the structure of the embryo and endosperm at maturity have been studied by Weatherwax and a number of other workers, but relatively little attention has been devoted to the important intermediate stages. True (*47*), Guérin (*15*), Poindexter (*33*), Weatherwax (*52*), Randolph (*34*), and Haddad (*19*) traced the histological changes which take place during the transformation of the ovary wall into the pericarp of the mature grain. The presence at maturity of a testa consisting of integument tissue was described by Weatherwax, and True also indicated that both integument and nucellar remnants were present in the fully developed kernel. However, Guérin, Poindexter, and F. R. Randolph found little or no trace of either integument or nucellar tissue in the ripe grain.

The existence of a specialized semipermeable "seed-coat membrane" in the maize caryopsis was suggested by the physiological researches of Shull and Shull (*40*), Beeskow,[5] Orton (*32*), and Tharp.[6] Beeskow attributed this functional semipermeability in maize to several layers of chiefly disorganized cells between the pericarp and the endosperm. In the related grasses *Sorghum*, *Hordeum*, *Triticum*, and *Poa* the presence of a definite suberized semipermeable membrane has been demonstrated both physiologically and morphologically by Brown (*5, 6*), Harrington and Crocker (*20*), Andersen (*1*), Gurewitsch (*17*), and Krauss (*24*). In the present study a similar well-defined suberized layer was observed in maize, and its developmental history was carefully traced.

MATERIAL AND METHODS

The material for these studies was obtained from commercial varieties of maize grown under ordinary field conditions in the experimental garden of the Department of Plant Breeding at Ithaca, N. Y. The dent variety Pride of Michigan furnished most of the material, but other varieties of dent corn as well as sugary and flint types were examined. However, no significant morphological differences were noted among the different types other than variations which might readily be attributed to differences in the size, shape, or degree of maturity of the kernels.

To provide suitable material for a study of the time factor in relation to fertilization and kernel development, controlled pollinations were made, all of the plants for a given series of stages being hand-pollinated at one time. Thereafter samples were collected at the desired intervals. The age of the material in all cases was designated in terms of hours or days after pollination. Temperature relations were considered as an important factor influencing the rate of development, and seasonal variations also were studied in series of stages collected during three different growing seasons.

The method of preparing the material for microscopical study was as follows: Freshly harvested samples were fixed in a chromo-acetic acid mixture, containing 0.7 percent chromic acid and 1 percent glacial acetic acid, to 20 cc of which was added 3 drops of a 2-percent solution of osmic acid at the time of fixation. Paraffin sections 12μ to 15μ in thickness were prepared and stained, either with Flemming's triple stain or with Delafield's haematoxylin. Freehand sections of fresh and fixed material also were utilized, and in making determinations of the composition of the cell wall Sudan IV and certain other stains were employed.

DESCRIPTION

In the following account the essential features of the developmental morphology of the maize caryopsis are described. The investigation was concerned primarily with a study of embryogeny and the development of the endosperm and pericarp. However, an understanding of the changes which occur in the transformation of the ovary into the caryopsis requires a knowledge of the parts of the pistillate inflorescence which most directly are concerned in the development of the kernel. For this reason a brief description is first given of the mature pistillate spikelet, the ovary and ovule, and certain essential facts concerning pollination and fertilization. The structure of the entire caryopsis at different stages in development then is described, and this is followed by separate accounts of the developmental history of the embryo, endosperm, the integuments, the nucellar membrane, and the pericarp.

PISTILLATE SPIKELET

The morphology of the pistillate spikelet in maize has been described by Wigand (*55*), True (*47*), Guignard (*16*), Weatherwax (*50*), Miller (*30*), Stratton (*44*), and Arber (*2*). The spikelets ordinarily are borne in pairs arranged on the ear shoot or cob in double longitudinal rows. The total number of double rows on the ear usually ranges from four to eight, although higher numbers occur not infrequently. The individual spikelet normally has two flowers, of which the upper is functional and the lower aborted (fig. 1). But the reverse condition is sometimes found, especially near the tip of the ear. In kernels developed from the upper flower of the spikelet the embryo

FIGURE 1.—Mature pistillate spikelet in radial longitudinal section: gl_1 and gl_2, Glumes subtending the spikelet; fl_2, aborted second flower; p_2 and l_2, palea and lemma of aborted flower; l_1, st_1, p_1, lemma, stamen, and palea of fertile flower; es, embryo sac; nu, nucellus of ovule; ov, ovary wall; s, style; sc, stylar canal.

is on the side of the kernel toward the tip of the ear. When the lower flower develops the embryo is on the side of the kernel toward the base of the ear.

A number of interesting special cases have been reported in which both flowers of the spikelet develop, producing paired grains, which are completely separate from one another, or double or connate grains resulting from some degree of fusion of the two flowers. In either case the members of the pair are arranged back to back, with the embryo of the upper grain on the side toward the tip of the ear and the embryo of the lower grain on the side toward the base of the ear.

Paired grains were first described in pod corn by Sturtevant (*45*), who noted exceptional instances of twin seeds in individual pods. Later, Kempton (*22*) reported a number of cases of inverted grains and was the first to interpret correctly their mode of origin. In Country Gentleman sweet corn the development of both flowers of the spikelet is the prevalent condition and is responsible for the crowded, irregularly spaced grains which characterize this variety (*45, 49*).

Connate grains, made up of two kernels partially or completely united and enclosed in a common pericarp, were discovered by Kempton (*22*) in Hopi maize and in a hybrid between certain Chinese and Central American varieties. A strain of maize with many double and occasionally with triple grains, described by Blaringhem (*4*) as a new variety, *Zea mays* var. *polysperma*, was studied in the developmental stages by Stratton (*44*). It was found that in many instances both flowers of the spikelet were functional. Some developed separately as paired grains. Others fused more or less completely to form semiconnate or connate grains. Kernels of these types are compound structures, with embryos on opposite sides, and are not to be confused with simple grains having paired embryos on the same side of the kernel.

Another type of anomalous kernel development is represented by paired or twin embryos arranged side by side in an otherwise normal kernel. Such grains are found not infrequently in different stocks of maize, and the normal frequency of occurrence of this type of twinning is markedly increased by X-ray treatment. Since the pericarp and endosperm of these grains show no evidence of doubleness, the twin embryos presumably arise from a single embryo sac. Twin embryo sacs would be expected to produce twin endosperms as well as twin embryos. Furthermore, the twin plants produced by these kernels ordinarily are genetically identical, even in extremely heterozygous stocks, which indicates that they arise from a single zygote, presumably by a subdivision of the entire embryo or of that portion of it from which the plant develops, at some relatively early stage in ontogeny. Seedlings with two plumules and a single primary seminal root undoubtedly owe their origin to an incomplete separation of the embryo into two parts, a separation which involves only the portion which forms the plumule meristem.

The two flowers of the pistillate spikelet are partly enclosed by bracts, including a pair of empty glumes, and a lemma and palea associated with each flower in the positions indicated in figure 1. The upper fertile flower is essentially terminal with reference to the spikelet axis or rachilla. However, a rudimentary rachilla may be present, extending beyond the insertion of the fertile flower and between the paleae of the fertile and the sterile flower (*2*).

PISTIL

The functional pistillate flower in maize normally has a single pistil and three rudimentary stamens.[7] The pistil terminates in an elongated "silk", which properly may be referred to either as a stigma or a style, since it is receptive throughout its entire length but normally receives the pollen only at the tip. The basal portion of the pistil consists of a unilocular ovary containing a single highly specialized semianatropous ovule.

With respect to the extreme length of the style or silk, maize is unique among flowering plants. Ordinarily, the silk ranges in length from 10 to 30 cm, depending on the length of the ear shoot and the husks that envelop the ear and also upon the promptness with which pollination takes place after the silks are exposed. Normally, the silk ceases to elongate within approximately 24 hours after pollination, but, if pollination is deferred, elongation may continue for as long as a week or more and silk lengths of 50 to 75 cm may be attained under conditions favorable for growth.

The silk is branched at the tip and bears multicellular stigmatic hairs throughout its entire length. These hairs are very numerous at the tip and become progressively less numerous toward the base, where they are widely scattered. The silk is receptive throughout its entire length,[8] and normally remains so for a week or 10 days after it is first exposed.

The question of the fundamental structure of the pistil in *Zea* and other grasses has engaged the attention of morphologists for many years. The view that the grass pistil was derived from a single carpel was held by many early morphologists, including Schleiden (*37*), Hackel (*18*), and Engler (*11*), and is still adhered to by some modern investigators (*3*). Other early workers, including Roeper (*35*), Doell (*8*), Goebel (*13*), and Čelakovský (*7*), supported the view that three carpels are involved in the formation of the grass pistil, and this latter view is now generally accepted (*2*). The morphology of the maize pistil has been described by Schleiden (*37*), Wigand (*55*), Eichler (*9*), Poindexter (*33*), Walker (*48*), Schuster (*39*), Weatherwax (*53*), Arber (*2*), and others, and the tricarpellate nature of the pistil has been emphasized by most of these workers.

According to the interpretation of Schuster, the unilocular maize ovary consists fundamentally of three carpels united edge to edge. The single ovule is inserted near the base of the ovary on the side adjacent to the spikelet axis or rachilla in the region which represents, fundamentally, the fused margins of the two lateral carpels. The midrib bundles of the lateral carpels pass into the silk, and the fused marginal bundles of these lateral carpels supply the ovule. The third carpel is rudimentary, but a vestigial vascular trace, interpreted as the median bundle of this carpel, is sometimes present at the base of the ovary directly opposite the insertion of the ovule. The single style is obviously a double structure, presumably formed by a fusion of the styles of the two lateral carpels (*14*). This view is supported by the fact that the style is branched at the tip and has throughout its length

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[7] In certain types of maize, notably dwarf and anther-ear types, fully developed stamens are present in both flowers of the pistillate spikelet (*10*).
[8] It is noteworthy in this connection that Mangelsdorf and Reeves (*29*) were successful in obtaining hybrids between *Zea* and *Tripsacum* by applying *Tripsacum* pollen directly to the base of maize silks after pollinations made in the usual manner had failed repeatedly to set seed.

a median constriction and that it has two vascular bundles with each of which is associated a strand of tissue interpreted as remnants of locular extensions of the two lateral carpels (*34*).

OVARY AND OVULE

The ovary at the time of fertilization is roughly spherical except in the region of the silk attachment, where it is somewhat elongated (fig. 2). Just below the region of silk attachment, on the side of the ovary away from the ear-shoot axis, there is a slight protuberance which marks the position of the stylar canal, so called by Guignard (*16*), who believed, wrongly, that the canal served to conduct the pollen tubes to the ovule. It is in this stylar-canal region that the margins of the ovary wall unite in ontogenetic development to enclose the ovarian cavity. The ovary wall at this stage is made up chiefly of thin-walled parenchyma cells capable of rapid growth by division and enlargement. The vascular tissue entering the base of the ovary supplies the ovule and subdivides to form the two prominent bundles of the silk and a varying number of rudimentary bundles in the lower portion of the ovary.

FIGURE 2.—Mature ovary in radial longitudinal section: *s*, Base of style; *sc*, stylar canal; *es*, embryo sac; *nu*, nucellus; *ii*, inner integument; *oi*, outer integument; *ov*, ovary wall.

The maize ovule at maturity is relatively large and virtually fills the ovarian cavity, as indicated in figure 2. In form it is definitely unlike any standard type, and represents an extremely modified condition variously referred to as semianatropous or modified campylotropous. The ovule has two integuments, a very broad insertion region, and no well-defined funiculus. The inner integument completely surrounds the ovule except at the micropylar orifice. The outer integument does not completely surround the ovule, being absent in a limited oval-shaped area extending from the micropyle in the direction of the silk-attachment region to the crest of the ovule. The embryo sac occupies a relatively small part of the nucellus in the micropylar region of the ovule.

POLLINATION AND FERTILIZATION

The sequence of events relating to the fertilization process in *Zea* from the time of pollination to the initiation of embryogeny and endosperm development has been described by Guignard (*16*), Weatherwax (*51*), Miller (*30*), and Rhoades,[9] and the main features of the process are well known. A consideration of the cytological details of fertilization was outside the scope of this investigation, but the time factor in relation to syngamy and the beginning of kernel development was carefully studied.

The pollen germinates almost immediately after it reaches the silks, as determined by direct microscopic examination of freshly ex-

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[9] RHOADES, V. H.　See footnote 4.

cised silk tips immediately after pollination. Within 5 minutes after the pollen was applied to the silks the pollen tubes emerged and were seen entering the central core of the multicellular stigmatic hairs, through which they passed into the main portion of the silk. In the early stages of pollen-tube growth, active protoplasmic streaming was noted repeatedly; the granular elements in the cytoplasm were seen to pass to and fro in the pollen tube and from the pollen grain into the pollen tube and back again. As the growth of the pollen tube continued, more and more of the granular material originally present in the pollen grain became localized in the pollen tube, and after 3 or 4 hours most of this granular material was no longer present in the pollen grain. The passage of the male gametes from the pollen grain into the pollen tube ordinarily takes place from 2 to 4 hours after pollination, and their movement into and along the pollen tube apparently is aided by the protoplasmic streaming. These observations were made at temperatures ranging from 22° to 25° C. At lower temperatures somewhat slower rates were noted, and at higher temperatures the rates were somewhat faster. These results are not in agreement with those of Miller and of Weatherwax, who stated that a few hours or at least 2 hours were required for germination to take place. However, Rhoades [10] and Sprague (*42*) reported that germination takes place soon after the pollen grains lodge on the silks.

The time interval between pollination and fertilization was found by Weatherwax (*51*) to be approximately 25 hours for silk lengths of about 25 cm. Miller (*30*) observed that ovules with silk lengths as great as 6 inches had been penetrated by the pollen tubes within 24 hours after pollination under ordinary field conditions. In plants maintained at a constant temperature of 25° C., Rhoades noted that the interval from pollination to fertilization was about 15 hours for silk lengths of 7 to 11 cm and about 17 hours for silk lengths of 11 to 14 cm. In the present investigations it was observed that in plants grown under field conditions at Ithaca, N. Y., with maximum day temperatures of 25° to 30° and minimum night temperatures of 13° to 17°, the interval was approximately 16 hours for silk lengths of 3 to 5 cm and 23 hours for silk lengths of 15 to 18 cm.

The fertilization of the egg and the polar nuclei by the male gametes takes place simultaneously, or nearly so, very soon after the pollen tube enters the embryo sac. An interval of 10 to 12 hours ordinarily intervenes between the fertilization of the egg and the first division of the zygote. The primary endosperm nucleus, however, undergoes division almost immediately after fusion is completed, and there are usually four or eight free endosperm nuclei present in the embryo sac when the fertilized egg divides.

TRANSFORMATION OF PISTIL INTO CARYOPSIS

The morphology of the pistil is profoundly altered during the development of the caryopsis. The ovule and its integuments are displaced by the developing embryo and endosperm, and there is little resemblance between the pericarp of the mature kernel and the ovary wall from which it was derived. The shape as well as the size of the caryopsis at maturity is very different from that of the pistil in the initial stages of kernel development. Before proceeding to give

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[10] Rhoades, V. H. See footnote 4.

separate detailed accounts of the developing embryo, endosperm, and pericarp, the major morphological changes involved in the transformation of the pistil into the mature caryopsis will be outlined briefly.

The ovary in the initial stages of kernel development is nearly spherical in outline, and consists chiefly of thin-walled parenchyma cells capable of rapid growth. Within 3 or 4 days after pollination [11] a several-celled embryo embedded in cellular endosperm is well established in the nucellar tissue near the base of the ovule (fig. 3, *A*). At this stage the endosperm occupies an area not much greater than the space originally occupied by the embryo sac. The integuments
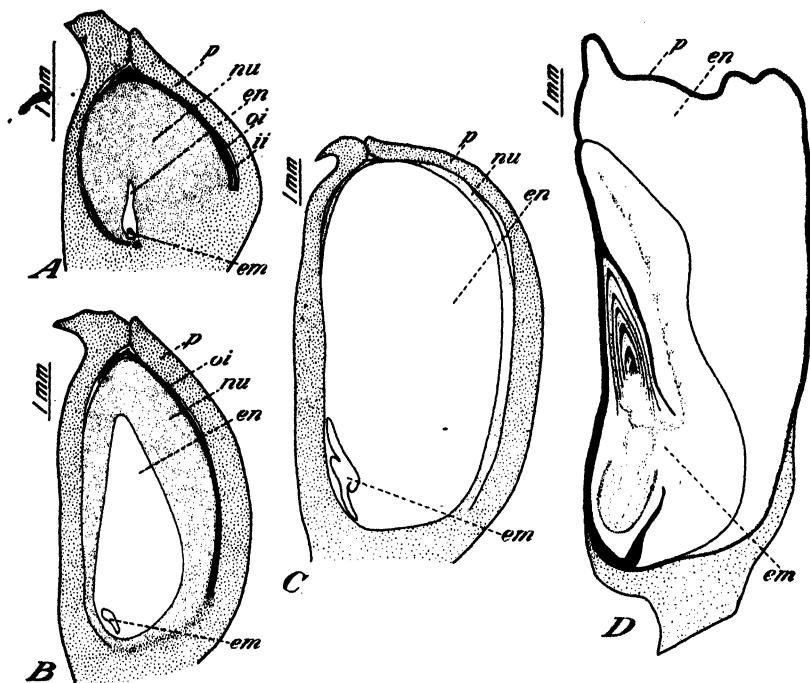
FIGURE 3.—Developmental stages in the transformation of the pistil into the caryopsis: *A*, 4 days after pollination; the embryo and endosperm have started to develop, but the structure of the ovary is essentially the same as at the time of fertilization. *B*, 10 days after pollination; the young caryopsis has enlarged considerably, the integuments are disappearing, and the endosperm is rapidly digesting away the nucellus of the ovule. *C*, 18 days after pollination; the caryopsis has continued to enlarge, the integuments have virtually disappeared, the nucellus is in the final stages of disappearance, and the compression of the pericarp in the crown region has begun. *D*, Morphologically mature kernel 45 days after pollination, consisting essentially of embryo, endosperm, and pericarp. The aleurone layer at the periphery of the endosperm is shown as a dotted line just inside the pericarp. The nucellar membrane between the aleurone layer and the pericarp is not shown in this drawing. *p*, Pericarp; *nu*, nucellus of the ovule; *oi*, outer integument; *ii*, inner integument; *en*, endosperm; *em*, embryo.

of the ovule are essentially intact, although disintegration of the inner integument has begun along the germinal face of the ovule. In this early period, from 2 to 5 days after pollination, there is a considerable enlargement of the entire ovary, unaccompanied by any appreciable change in form.

[11] The rates of development referred to throughout the paper are those which were typical of material collected under field conditions at Ithaca, N. Y., in 1924, and are based upon the examination of several kernels from each of 52 fixations taken at different intervals from fertilization to maturity. The ear shoots were hand-pollinated on Aug. 16 and the kernels were fully mature on Oct. 5. The rate for this particular season was intermediate between a somewhat more rapid rate of maturity noted during 1923 and a somewhat slower rate during 1926.

The period from 5 to 10 days after pollination is characterized by a pronounced elongation of the young caryopsis and by a rapid growth of the endosperm. A 10-day stage is illustrated in figure 3, *B*. The ovary wall has increased rapidly in length and to a lesser extent in width. Scattered nuclei surrounded by remnants of cytoplasm are all that remain of the inner integument over the germinal face of the ovule, but at the top and over the abgerminal face the inner integument is still intact. The outer integument is starting to disintegrate at the abgerminal attachment region and in the region of the stylar canal. The embryo is still relatively small, but the endosperm has expanded until it occupies approximately one-half of the nucellus, which itself is about twice as wide and three times as long as it was at the time of fertilization.

The growth of the entire kernel continues at a very rapid rate during the 10- to 20-day period, as shown by the following data from measurements of the length of the caryopsis taken at 5-day intervals from the time of pollination to maturity:

Age of material (days):	Length of kernel (millimeters)
0	1. 5
5	2. 5
10	4. 5
15	6. 5
20	8. 0
25	9. 0
30	10. 0
35	11. 0
40	11. 5
45	11. 5
50	11. 5
55	11. 0

The endosperm increases several times in volume and in so doing encroaches upon and digests away the nucellus, which has been displaced, except in a very limited peripheral area, at the end of 18 days (fig. 3, *C*). At this stage the integuments, except at the stylar-canal region, have disappeared as definite layers of tissue, leaving here and there only scattered masses of disintegrating protoplasts between the inner epidermis of the pericarp and the outer epidermis of the nucellus. The pericarp has now reached its maximum thickness, and in the crown region a collapse of the inner cells has taken place, a process which later extends throughout the extent of the pericarp and partly accounts for the very appreciable reduction in thickness as maturity is reached. In the embryo the main plumule-radicle axis is fully differentiated, but the rate of growth of the embryo during this period is relatively slow in comparison with the much more rapid growth of the endosperm.

During the latter part of the developmental period in the formation of the caryopsis the most conspicuous morphological changes are an extensive enlargement of the embryo, further increase in the size of the endosperm and the differentiation of its epidermis into the aleurone layer, the complete disappearance of the nucellus with the exception of the outer wall of the epidermal cells, and the reduction of the pericarp to a thin lignified layer surrounding the caryopsis (fig. 3, *D*). The growth of the embryo is most rapid from about 20 to 40 days after pollination (table 1). The enlargement of the endosperm and pericarp in the final stages is brought about chiefly by an increase in cell size;

cell-division activity is relatively slight after about 45 days. The caryopsis attains its maximum size at 40 to 45 days and is morphologically mature, except for some further growth of the embryo. At this time the grain passes from the so-called "soft-dough" to the "hard-dough" stage. After approximately 45 days the growth of the embryo normally ceases.[12] During the next 5 or 10 days the crown region of the kernel becomes indented and by the end of 50 or 55 days the caryopsis has assumed the typical mature form.

TABLE 1.—*Rate of growth of the embryo as determined by length measurements during development*

Age of embryo (days)	Length of embryo	Increase in length	Age of embryo (days)	Length of embryo	Increase in length	Age of embryo (days)	Length of embryo	Increase in length
	Mm	*Mm*		*Mm*	*Mm*		*Mm*	*Mm*
5	0.1		20	2.7	1.3	50	8.1	1.6
10	.4	0.3	30	4.5	1.8	55	8.4	.3
15	1.4	1.0	40	6.5	2.0			

EMBRYOGENY

The development of the embryo in maize has received little attention. Weatherwax (*52*) outlined the course of development very briefly. Other workers have concerned themselves chiefly with the mature embryo for the purpose primarily of determining the homologies of the scutellum, coleoptile, nodes, etc., in relation to those of other grasses. An extensive critical review of the literature has been prepared,[13] and a summary of the various conflicting views pertaining to this highly controversial subject has been published, by McCall (*26*). The present account is limited to a consideration of the ontogenetic development of the embryo.

The two-celled embryo, formed by the division of the fertilized egg or zygote (fig. 4, *B*), consists of a small lenticular apical cell and a much larger basal cell (*C*). This division of the zygote occurred from 30 to 34 hours after pollination under the conditions that prevailed during the growth period of the material used in these studies, i. e., with maximum day temperatures of 25° to 30° C., minimum night temperatures of 13° to 17°, and silk lengths of 12 to 18 cm. In plants maintained at a constant temperature of 25° and with silk lengths of 10.5 to 14.5 cm, Rhoades [14] obtained two-celled embryos 28 to 30 hours after pollination. In the present study embryos with three or four cells were noted at 36 hours (*E–G*), and at 42 hours the apical cell ordinarily had divided twice, and two or three divisions had occurred in the subapical portion (*H–N*). Four days after pollination the embryos contained ordinarily from 10 to 24 cells (*O–Q*).

Especially noteworthy in connection with the early history of the maize embryo is the fact that the proembryo develops very irregularly with no very definite or orderly arrangement of the cells or sequence of cell divisions. In most grass embryos previously described, it has
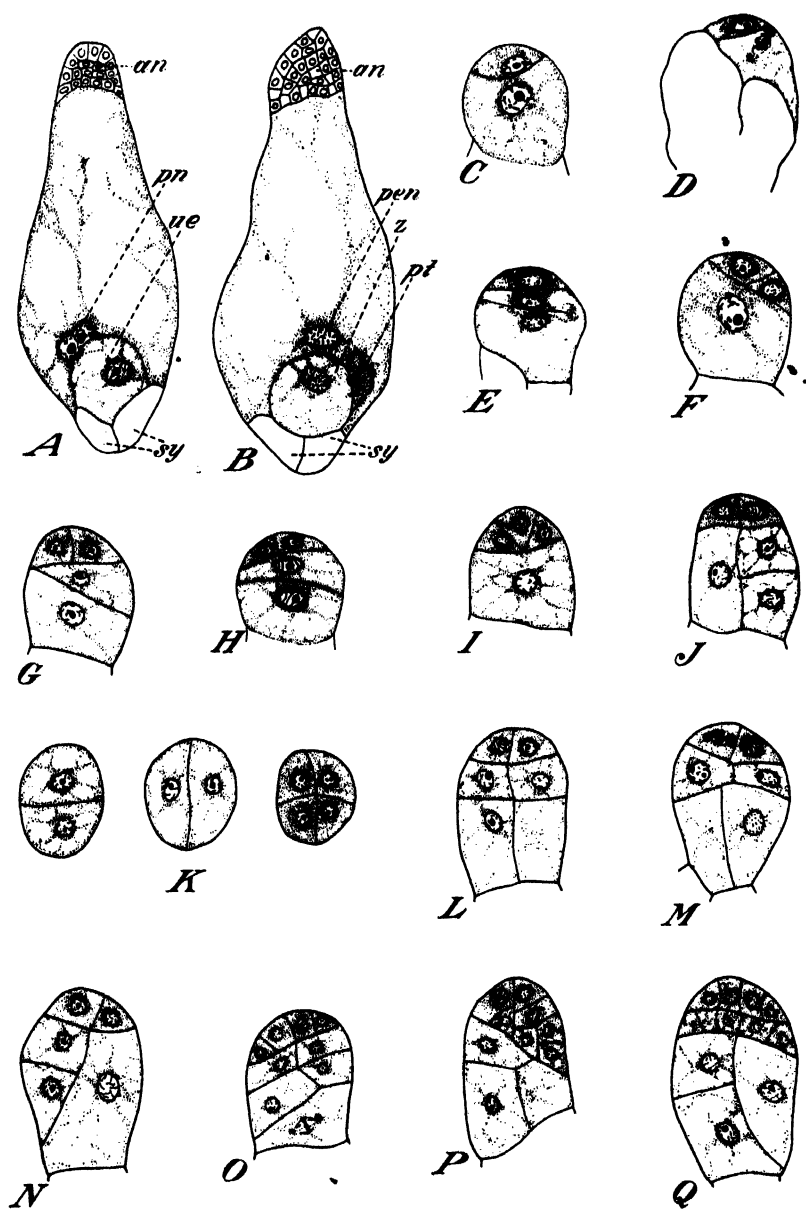
FIGURE 4.—Embryo sacs before and after fertilization, and initial stages in the development of the pro-
embryo illustrating the varying position and sequence of the cell walls. *A*, Embryo sac before fertiliza-
tion: *an*, Antipodals; *pn*, polar nuclei not yet fused; *ue*, unfertilized egg; *sy*, synergids. *B*, Embryo sac
after fertilization: *an*, Antipodals; *pen*, primary endosperm nucleus; *Z*, zygote (fertilized egg); *pt*, pollen-
tube remnant; *sy*, synergids. *C* and *D*, Two-celled proembryos 32 hours after pollination. *E–G*, Three-
and four-celled stages 36 hours after pollination. *H–N*, Stages observed 42 hours after pollination. *O–Q*,
Proembryos with 10 to 24 cells, 4 days after pollination. All figures are from radial longitudinal sections
except those of *K*, which are cross sections from the base to the tip of a stage similar to *H*, *A*, *B*, × 200;
C–H × 225.

been stated that tiers of three or four cells with parallel cross walls are formed in a regular manner, and that there is a very definite sequence of cell divisions in the initial stages (*3, 38, 41*). In *Zea,* fairly regular tiers of cells occasionally are produced (fig. 4, *E, H, O*), but more frequently the arrangement is quite irregular (*G, I, P*) and the order in which the cells divide is inconstant. The proembryo of maize does not regularly conform to any definite growth pattern.

During the period from 4 to 8 days after pollination the growth of the proembryo is limited chiefly to the apical region (fig. 5). In the basal or suspensor region few cell divisions occur. As a result of the relatively much greater cell-division activity in the upper portion, the embryo develops into a club-shaped structure (fig. 5, *C–H*). The original cell boundaries, which at an earlier stage subdivided the embryo longitudinally into three or four parts of unequal size, may be identified readily as late as 4 to 6 days after pollination (*A–C*). Thèreafter, they are identified with difficulty or not at all, and it is impossible to detect in the later stages the regions that originated
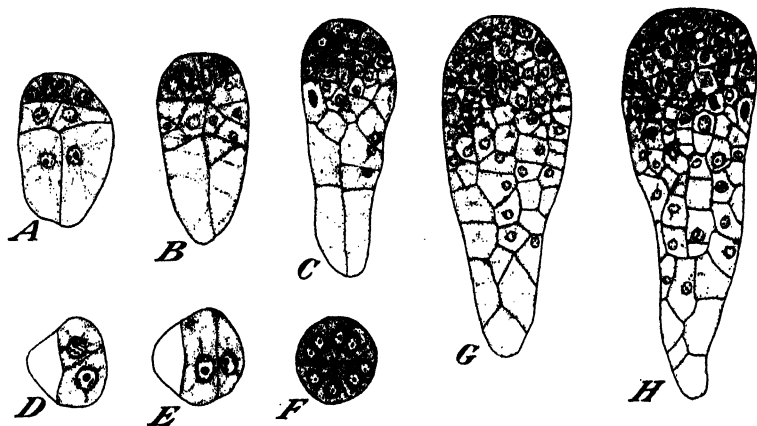
FIGURE 5.—Proembryos 4 to 8 days after pollination. In radial longitudinal section: *A*, 4 days; *B*, 5 days; *C*, 6 days; *G*, 7 days; *H*, 8 days. *D–F*; Transverse sections from the base to the tip of a 4-day embryo. *A–C, G, H* × 225.

from any of the original sectors of the very young embryo. Except for the localization of growth, primarily in the upper portion, the proembryo is wholly undifferentiated up to and including the seventh day (*G*); at the periphery of the embryo both anticlinal and periclinal walls are formed, and elsewhere cell divisions occur at random without definite orientation of the daughter cells. A pronounced decrease in size accompanies the rapid increase in the number of cells during this period, and the relatively large, irregularly shaped cells of the 5-day embryo are replaced subsequently by smaller isodiametric cells.

Beginning approximately 8 days after pollination, differentiation progresses rapidly in the embryo. At this time a definitive epidermis makes its appearance over the apical region (fig. 6, *A*); later it extends gradually down the sides of the embryo toward the region occupied by the suspensor (*B*). Cell-wall formation becomes chiefly anticlinal in the epidermal tissue as differentiation progresses, and the production of periclinal walls, noted frequently in the peripheral
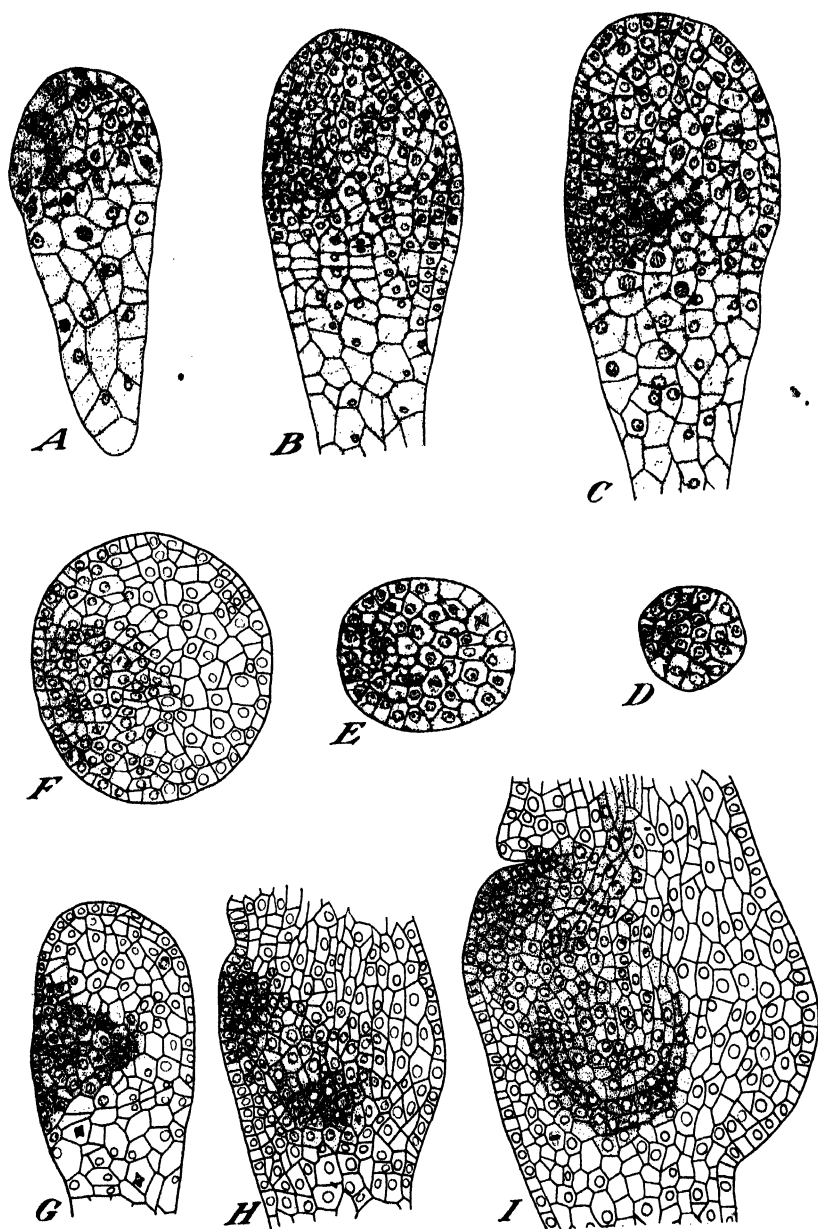
FIGURE 6.—Embryos 8 to 13 days after pollination, showing stages in the differentiation of the epidermis and the permanent plumule-radicle axis of the more mature embryo: *A-C*, Radial longitudinal sections showing stages at 8, 9, and 10 days after pollination; epidermis differentiating over the tip of the embryo in *A* and *B*. *C*, Initial stage in the differentiation of the plumule-radicle axis as a wedge-shaped group of cells in the anterior portion of the embryo. *D-F*, Transverse sections in the subapical region of embryos, 6, 7, and 10 days after pollination, showing stages in the differentiation of the epidermis and axis of the more mature embryo. *G-I*, Radial longitudinal sections of the subapical portion of embryos, 10, 12, and 13 days after pollination, illustrating in *H* and *I* the manner in which the stem and root portions of the plumule-radicle axis become differentiated from the single group of meristematic cells shown in *G*. *A-F*, × 200; *G-I*, × 175.

cells during the earlier stages (*A*), becomes relatively infrequent as the embryo matures. Another type of differentiation observed at about this stage consists in a marked tendency for the cells in the subapical region of the proembryo to divide actively, with the new cell walls forming at right angles to the axis of the embryo (*A*). Groups of cells with parallel cross walls are thus formed between the suspensor region and the tip of the embryo (*B*). In this manner the longitudinal extent of the meristematic tissue in the upper portion of the embryo is materially increased. This is obviously a preliminary step in the differentiation of the main axis of the more mature embryo, an axis which does not coincide with the axis of the proembryo.

The axis of the more mature embryo is first recognizable 9 or 10 days after pollination as a group of densely protoplasmic and actively dividing cells at the front (anterior face) of the embryo, slightly below the tip. This group of cells is roughly triangular when viewed either in radial longitudinal section (fig. 6, *C*), or in cross section (*F*). The rate of growth within this group of cells is more rapid than in the other parts of the embryo, and as a result a slight protuberance soon develops on the anterior face of the embryo (*G, H*), a stage which is ordinarily reached approximately 10 days after pollination. At 12 days this protuberance is noticeably enlarged (*H*). At 13 days it has increased still further in size, and the single group of meristematic cells previously formed has now differentiated into two distinct groups (*I*). Of these, the upper group with reference to the embryo axis is closely associated with the outgrowth or protuberance on the face of the embryo and is the primordium of the stem portion of the new axis; the lower group is the primordium of the corresponding root portion. Associated somewhat more closely with the former than with the latter primordium is a strand of elongated cells, which is the forerunner of the main vascular supply to the scutellum. Within the lower or root primordium certain cells very early assume a definite orientation, anticipating the delimitation of the coleorhiza and primary seminal root.

A clearer picture of the relative position of the differentiating axis of the more mature embryo in relation to the axis of the proembryo may be obtained from the illustrations in figure 7, *A–D*, which are reproduced at a relatively low magnification and show the form of the entire embryo at 10 to 14 days. This differentiating axis is a lateral structure oriented in an oblique position with reference to the longitudinal axis of the embryo (*B–D*). With the appearance of the new axis there is a pronounced shift in the polarity of the embryo, from an essentially vertical to a definitely oblique orientation, and the major growth activity during the later stages of embryogeny is centered about the differentiating plumule-radicle meristem. However, there is also an appreciable amount of growth in the suspensor region, particularly at 13 and 14 days (*C, D*); and in connection with the development of the scutellum there is a gradual and extensive enlargement both in the region which was formerly the tip of the proembryo and along the posterior side above the suspensor region. Figure 7, *C* and *D*, in comparison with *B*, shows the extent of growth during those stages in the suspensor and scutellum regions.
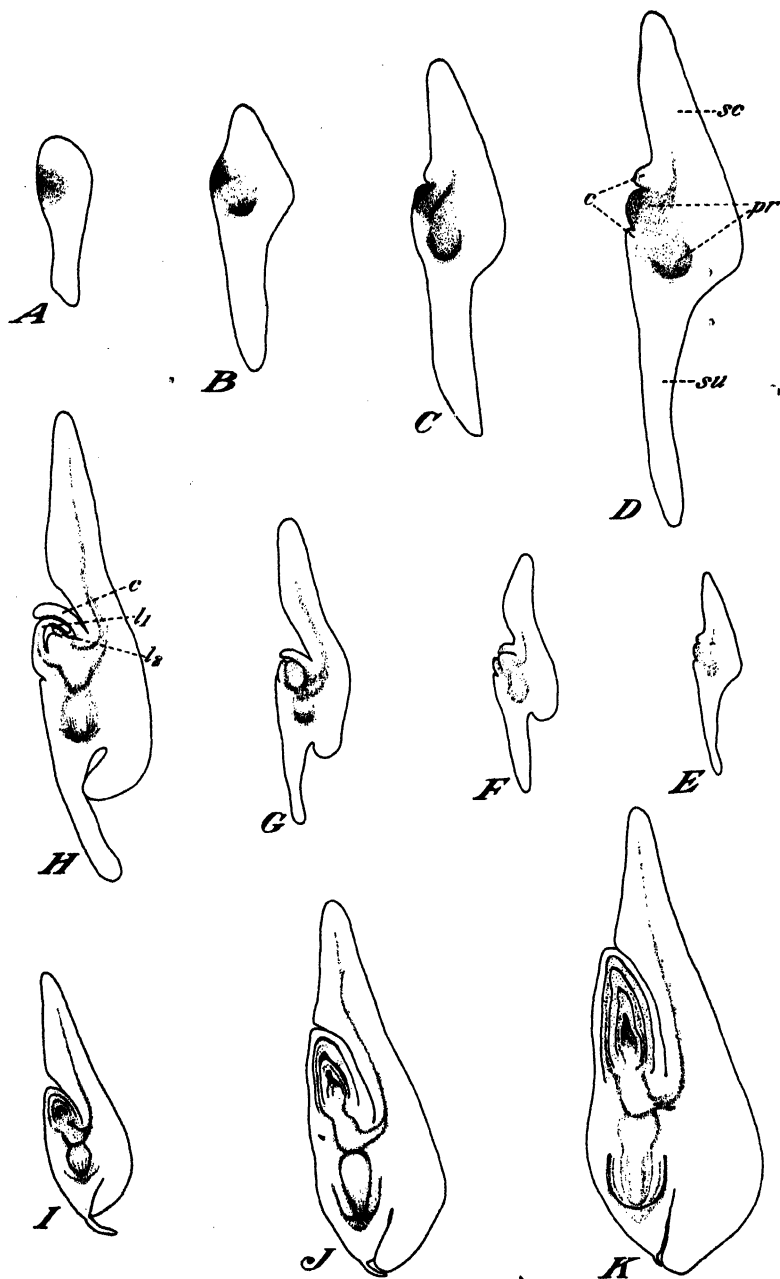
FIGURE 7.—Outline drawings of embryos in radial longitudinal section, 10 to 45 days after pollination, showing changes in shape and size and relative positions of different parts: *A–D*, Stages at 10, 12, 13, and 14 days, showing position of the differentiating plumule-radicle axis with reference to the suspensor and differentiating scutellum: *sc*, Scutellum; *pr*, plumule-radicle axis; *su*, suspensor; *c*, coleoptile primordium. *E–H*, Stages at 14, 16, 18, and 20 days after pollination, showing continued development of the axis and the scutellum, relatively little further growth of the suspensor, and the appearance of the primordia of the first seedling leaves: *c*, Coleoptile; *l₁* and *l₂*, primordia of first and second seedling leaves. *I–K*, Stages at 25, 35, and 45 days after pollination; the plumule-radicle axis and the scutellum become much enlarged, the suspensor persists merely as a vestigial organ, and additional seedling leaves appear; *K*, essentially mature embryo. *A–D*, × 60; *E–H*, × 25; *I–K*, × 12.

During the period from 14 to 20 days after pollination the primordia of the coleoptile and first seedling leaves make their appearance (fig. 7, *E–H*). The coleoptile is formed initially as a ridge of tissue which nearly surrounds the central stem meristem (*D, E*). This ridge develops more rapidly above than below the central meristem and at a relatively early stage assumes the form of a sheathing structure that envelops the stem tip and the embryonic seedling leaves. The first seedling leaf arises below the stem meristem as a ridge of tissue similar to and opposite that of the coleoptile primordium, and the second leaf appears subsequently above the stem meristem directly opposite the first leaf (*H*).

Additional seedling leaves continue to form during the period from 25 to 45 days after pollination, the total number present at maturity usually being five, and the radicle with its ensheathing coleorhiza becomes fully differentiated during this period (fig. 7, *I–K*). There is also a general enlargement of the entire plumule-radicle axis and a corresponding increase in the dimensions of the scutellum. The suspensor ceases to enlarge after 20 days and thereafter persists apparently as a nonfunctional and unimportant part of the maturing
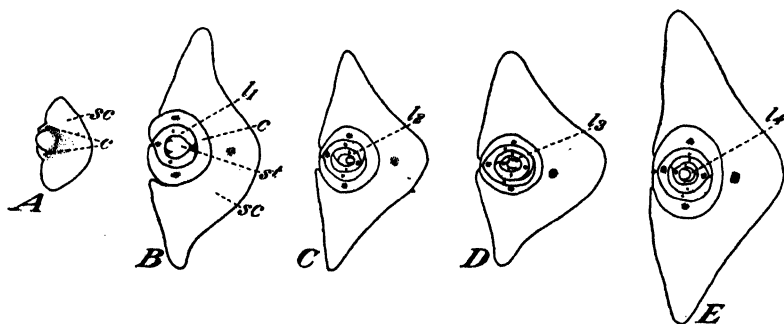
FIGURE 8.—Cross sections in the stem-tip region of embryos: *A–E*, 16, 20, 25, 35, and 45 days after pollination: *sc*, Scutellum; *c*, coleoptile; *st*, stem tip; *l₁–l₄*, successive seedling leaves; *A, B*, × 25; *C–E*, × 12.

embryo (*I–K*). As previously stated, the growth of the embryo as a whole is very rapid from 20 to 40 days after pollination, and the drawings *I* to *K*, which are of the same magnification, graphically illustrate this fact.

The relative position of the axis of the older embryo changes gradually in the later stages of maturity. At first the axis is definitely oblique (fig. 7, *D*), but later it assumes a position nearly parallel to the remainder of the embryo (fig. 7, *K*). It is also noteworthy that the scutellum essentially surrounds the axis of the embryo at maturity. This relationship is clearly shown in cross sections of the embryo at the level of the stem tip (fig. 8, *A–E*). These cross-section views also show the relative positions of the coleoptile and plumule leaves and the main vascular bundles supplying these structures and the scutellum.

The embryo is morphologically mature approximately 45 days after pollination, except for some additional development of the seedling leaves, of which there are normally five present in the ripe grain, and a slight increase in size throughout the embryo. The vascular tissues become more fully developed in the final stages of embryo formation, and a specialized epidermal tissue forms along

the posterior face and at the base of the scutellum. This specialized tissue serves a haustorial and secretory function as the embryo and seedling obtain nourishment from the endosperm.

ENDOSPERM FORMATION

The development of the endosperm begins almost immediately after fertilization, as described by Guignard (*16*), Weatherwax (*52*), and Rhoades.[15] The primary endosperm nucleus (fig. 4, *B*), which
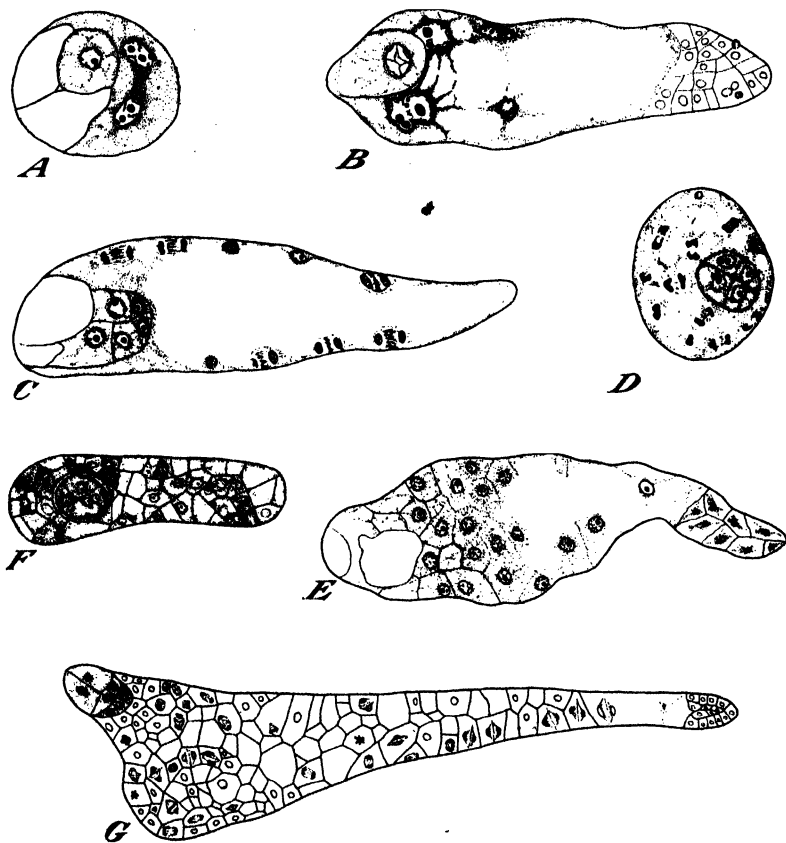
FIGURE 9.—Initial stages in the development of the endosperm: *A*, Cross section of the embryo sac at the level of the fertilized egg, showing the daughter nuclei formed by the division of the primary endosperm nucleus, 26 hours after pollination; *B*, longitudinal section of embryo sac 34 hours after pollination, showing zygote and eight-nucleate stage of free endosperm development, not all of the nuclei being shown in the figure; *C*, longitudinal section showing free endosperm nuclei in division, passing from the 128- to the 256-free-nucleate stage, 3 days after pollination; *D*, cross section of same stage as *C*; *E*, oblique longitudinal section of endosperm passing from free-nucleate to cellular phase, 3½ days after pollination; *F*, cross section of cellular endosperm in the region of the embryo, 4 days after pollination; *G*, median longitudinal section view at the same stage. *A-F*, × 175; *G*, × 80.

is formed by the fusion of the second male nucleus with one polar nucleus and the subsequent union of the product of this fusion with the second polar nucleus (*30*),[15] divides within 2 to 4 hours after the fusion is completed. The daughter nuclei then migrate to opposite sides of the embryo sac in the basal (micropylar) region adjacent to

[15] RHOADES, V. H. See footnote 4.

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the zygote (fig. 9, *A*). Within the 12-hour period following syngamy, i. e., within approximately 36 to 48 hours after pollination under the conditions which prevailed while these studies were in progress, a second, and frequently a third, division takes place, and the resulting four or eight free endosperm nuclei thus formed are distributed about the periphery of the micropylar end of the sac (*B*). Three days after pollination approximately 128 free endosperm nuclei are present, which is the number to be expected after seven nuclear divisions. Noteworthy in this connection is the fact that there is a very definite tendency for the divisions to occur in unison among the free endosperm nuclei (*C*), and this tendency persists even after the endosperm has become cellular (*G*). As the nuclei multiply, migration in the direction of the antipodal region of the sac continues and thus the entire embryo-sac cavity gradually becomes lined with nuclei.

The rapidity with which the endosperm begins to form is in marked contrast to the slower progress of embryogeny in the initial stages. Four to eight free endosperm nuclei are present at the time the fertilized egg divides, and when the embryo reaches the 6- to 8-cell stage there are from 128 to 256 free endosperm nuclei (fig. 9, *C*).

The developing endosperm passes gradually from the free-nucleate to the cellular phase, beginning about 3½ days after pollination. Cell-wall formation is initiated in the region of the endosperm surrounding the embryo and progresses in the direction of the antipodal cells (fig. 9, *E*). At 4 days the endosperm ordinarily is completely cellular except in the antipodal portion, and the middle region previously occupied by a large central vacuole becomes filled with cellular endosperm (*G*). When examined in cross-section view, that is, in a plane approximately at right angles to the axis of the proembryo, the endosperm in the micropylar region surrounding the embryo is at this stage very much flattened laterally and is only three or four cells in thickness (*F*). In the direction of the antipodals, however, the endosperm is much thicker.

The external form of the endosperm changes materially as it develops and there is also a tremendous increase in size. The length of the mature endosperm is approximately 50 times as great as it is in the initial stages and there is a corresponding increase in width. Before the histological features of growth and differentiation in the endosperm are described, the gross changes in external morphology will be outlined briefly.

The size and shape of the endosperm at different stages in development are shown in figure 10. In the early stages, up to 12 or 15 days, the enlargement of the endosperm is more rapid in the basal (micropylar) region than it is in the apical (antipodal) region (fig. 10, *A–F*). At 24 days the apical region is as large as or larger than the basal region (*G*), and in the later stages the former increases more rapidly than the latter.

The endosperm begins to elongate very rapidly, beginning 2 to 3 days after pollination (fig. 10, *C*). As this elongation takes place the antipodal cells retain their position at the apex of the endosperm tissue. As the endosperm increases in size the nucellar tissue immediately surrounding it is digested away and permits the growing endosperm to expand freely. At 2 days the approximate length of the endosperm is 0.3 mm, at 8 days 2 mm, at 24 days 10 mm, and at 48 days 15 mm; and there is a comparable increase in the diameter of the endosperm
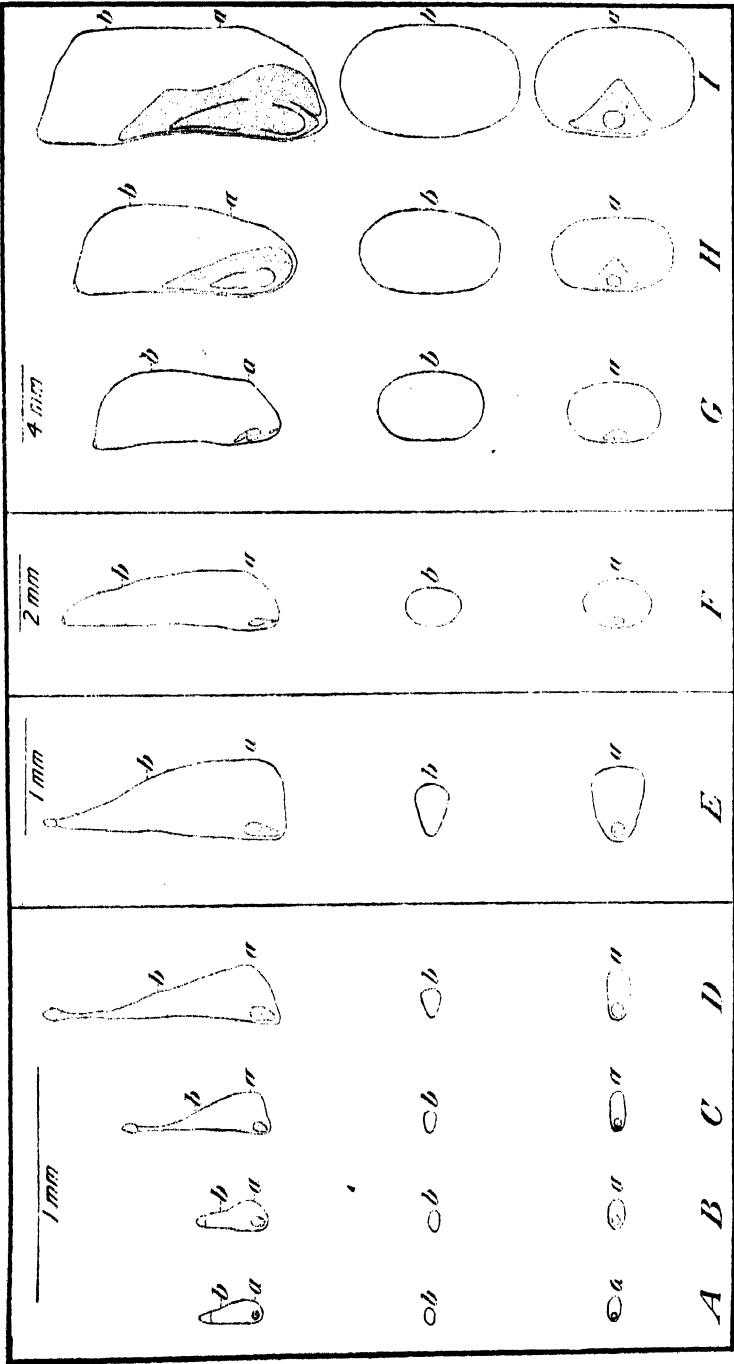
Figure 10.—Outline drawings showing changes in the size and shape of the embryo and endosperm at different stages of maturity; also position of the antipoda tissue at the tip of the endosperm in longitudinal sections. Radial longitudinal sections at top; cross sections below, at the level of the embryo (*a*) and at a level near the tip of the endosperm (*b*), as indicated in the longitudinal sections. The age of the different members of the series in days after pollination is as follows: *A* 1½; *B*, 2; *C*, 3; *D*, 4; *E*, 8; *F*, 12; *G*, 24; *H*, 36; *I*, 48.

during the same period. The dimensions of the endosperm in planes of section at right angles to the longitudinal axis are portrayed in the cross-section drawings of figure 10. One of the series of sections illustrated (series *a*) was from the level at the tip of the embryo and the other (series *b*) was from the region near the tip of the endosperm. The shape of the endosperm in cross section, as illustrated by these figures, is oval, or roughly so, in most stages of development. However, in the early stages the extent of the endosperm is much greater in the germinal-abgerminal direction than it is in the longitudinal plane at right angles to this (*A–F*), while in the later stages (*G–I*), because of the crowding of the kernels on the ear, this condition is reversed and the width is greater than the germinal-abgerminal thickness of the endosperm.

The internal differentiation of the endosperm was studied cytologically from the time it passed from the free nucleate to the cellular condition until it reached maturity, in order to determine as accurately as possible the manner in which growth and specialization take place within the endosperm. Just as in the case of the young embryo, the endosperm in the early stages is essentially undifferentiated and consists of thin-walled parenchyma cells of varying size and shape. There is at first no specialized epidermal layer and cell-division activity is general throughout the entire tissue, including both the marginal and the central cells (figs. 9, *G*, and 11, *A*). Gradually there is a tendency for cell division to be localized chiefly in the peripheral zone (fig. 11, *B–F*) and the growth of the inner region is primarily by increase in the size rather than in the number of cells.

There are several layers of cells in the peripheral region of the endosperm that are involved in the cell-division activity during the period from 12 to 16 days after pollination (fig. 11, *D–F*). In the epidermal layer of cells periclinal wall formation takes place repeatedly as development proceeds, and in the underlying cells additional divisions occur in the same plane. Rows of cells are thus formed which extend inward toward the central region of the endosperm (*E, I*). This arrangement of cells in fairly definite radial rows closely resembles the arrangement produced by cambial activity in the formation of secondary tissues.

The cambiumlike activity described above involved the epidermal layer of cells up to the 16- or 18-day stage along the germinal face of the endosperm (fig. 11, *G*), and for 3 or 4 additional days along the abgerminal face of the endosperm. In the crown region and in the basal region of the endosperm there was relatively little meristematic activity during this period; the cells in this region were relatively large and presented the appearance of a well-differentiated tissue. It is probable that this basal tissue functions actively in the transfer of nutrients from the vascular tissue of the caryopsis to the developing endosperm and embryo, as Weatherwax (*54*) and Lampe (*25*) have suggested. The cytological observations which were made to determine the manner of growth throughout the endosperm did not confirm the statement of Lampe (*25, p. 353*) that cell-division activity is more prolonged in the basal region than it is elsewhere. In fact, the reverse condition was noted in the different stocks examined, namely, that cell-division activity ceased first in the basal region and was much more prolonged at the periphery of the middle portion between

the base and the crown region of the endosperm. It is possible that the endosperm develops in a different manner in other varieties of maize.
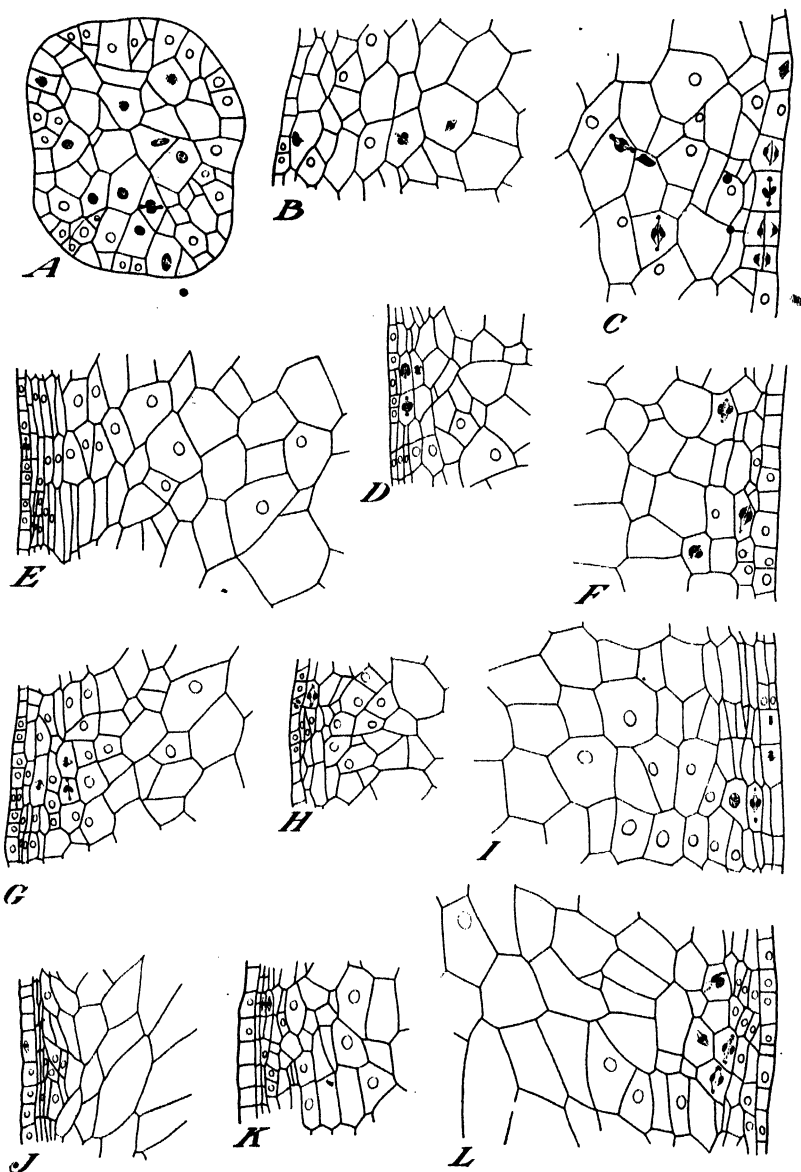
FIGURE 11.—Cellular structure of the endosperm from different regions of kernels at intervals of 6 to 21 days after pollination, showing manner of growth and progress of differentiation. *A*, Cross section from middle portion of the endosperm at 6 days. *B–F, G, I, J, L,* Radial longitudinal, and *H, K,* cross sections from germinal and abgerminal regions of the endosperm at a level just above the embryo; figures with the epidermal layer to the left are from the germinal portion and those with the epidermis to the right are from the abgerminal portion of the endosperm. The age of the endosperm in days after pollination is as follows: *B,* 9; *C,* 11; *D,* 12; *E* and *F,* 16; *G–I,* 18; *J–L,* 21. During the developmental period represented by these figures the epidermis of the endosperm, which later becomes the aleurone layer, is differentiated. × 125.

The epidermal layer of endosperm cells almost entirely ceases its meristematic activity in the germinal region approximately 18 days after pollination and at 20 to 22 days in the abgerminal region (fig. 11, *J–L*). This layer then assumes the characteristics of a distinct epidermal tissue; subsequent growth is almost exclusively by anti-clinal wall formation and by cell enlargement. This epidermis in the definitive state is the aleurone layer of the morphologically mature endosperm. Meristematic activity persists in the subepidermal tissue until about 45 to 48 days after pollination; the cells in this region remain relatively small in comparison with the much larger cells in the epidermal, or aleurone, layer, and cambiumlike rows of cells continue to form as late as 48 days after pollination in the anterior and posterior regions of the endosperm (fig. 12, *E* and *F*). In the final stages of endosperm development these subepidermal cells cease their division activity and increase markedly in size (fig 12, *G* and *H*). Occasional cell divisions occur in the aleurone layer, especially in the region extending over the face of the embryo, during the final stages of endosperm development.

ANTIPODAL TISSUE

At the time of fertilization in maize the embryo sac contains a varying number of antipodal cells, usually ranging from 24 to 48, grouped together in the end of the sac opposite the egg cell and the synergids (pl. 1, *A*). The antipodals are relatively small, chiefly uninucleate cells. They constitute a haploid tissue having the same genetic constitution as the egg cell, since both are derived from a single megaspore (*51*). In many grasses other than maize, the antipodals disintegrate soon after endosperm formation begins, and it was formerly believed that a similar type of behavior characterized the antipodals in *Zea* (*30, 51*). Later Weatherwax (*53*) reported that groups of antipodal cells had been observed in nearly mature kernels.

During the course of the present study, in which the development of the caryopsis was traced in a close series of stages, the presence of antipodal tissue was noted at all stages from fertilization to maturity. The antipodal cells not only persist but in many instances retain the capacity to divide, and as kernel development proceeds there may be an appreciable increase in the amount of antipodal tissue. Typical mitotic nuclear divisions were observed not infrequently, and in some of these the typical haploid number of 10 chromosomes was identified.

The frequency of occurrence of antipodal tissue in the advanced stages of kernel development was determined by a microscopical examination of serial longitudinal sections of caryopses from the commercial maize variety Pride of Michigan. Antipodal tissue was definitely identified in 28 of 52 caryopses examined. A similar examination of a smaller number of kernels from other varieties and genetic cultures suggested that there may be a considerable amount of variation in the frequency with which the antipodal tissue persists in different stocks of maize. An explanation of the absence of the antipodals in many nearly mature kernels was obtained by tracing their developmental history in the earlier stages of kernel development.

During the first 4 to 6 days after pollination the antipodals were present in all the ovules examined. Throughout this period they retained the same general appearance that they presented at the time of fertilization (pl. 1, *A* and *B*); there was little or no evidence
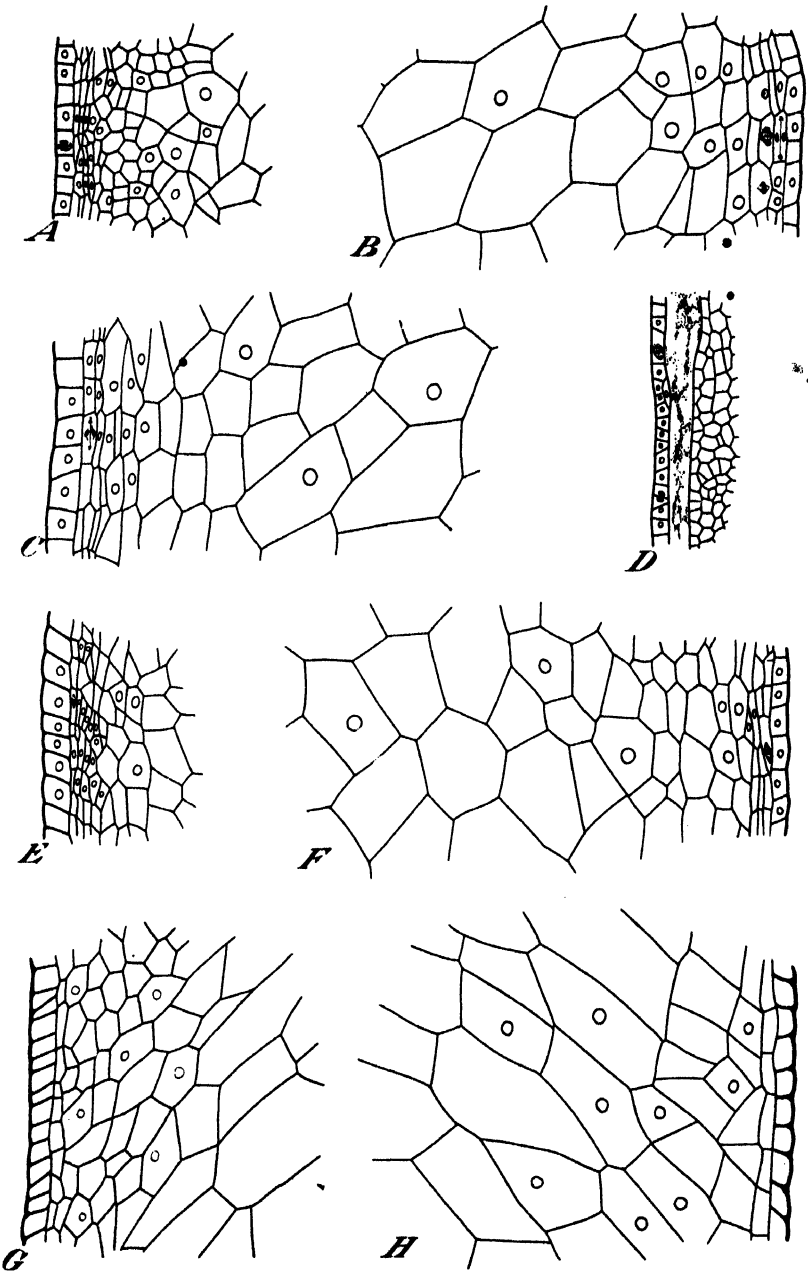
FIGURE 12.—Cellular structure of the endosperm as it approaches maturity, showing regions of the endosperm comparable to those in figure 11: *A* and *B*, 26 days; *C* and *D*, 36 days; *E* and *F*, 48 days; *G* and *H*, 55 days; in *D* the epidermal or aleurone layer of the endosperm is shown extending over the face of the scutellum tissue of the embryo. *A, B, E* are from cross sections and *C, D, F–H* are from radial longitudinal sections. × 125.

either of growth by cell division or of disintegration. However, the position of the antipodal tissue within the nucellus changed appreciably during this period, a change brought about by a translocation of the antipodals away from the micropylar region into the deeper lying tissues of the nucellus. This change in the position of the antipodal tissue occurred concomitantly with the development of the endosperm in the same general direction. The distance traversed by the antipodals during this period of migration was roughly equivalent to from 2.5 to 3 times the longitudinal extent of the embryo sac at the fertilization stage (cf. fig. 9, *B* and *G*).

The change in the position of the antipodal cells and the enlargement of the developing endosperm were facilitated by the disintegration of the nucellus adjacent to these tissues (pl. 1, *B*). The nucellar tissue is not crushed and compressed by the expanding endosperm and antipodals but becomes disorganized and gradually disappears, presumably being digested away and eventually resorbed through enzyme activity.

Initial stages in the disintegration of the antipodal tissue were noted at about 6 days after pollination (pl. 1, *C*), and more advanced stages were noted a few days later (*D*). Along with these cases in which disorganization of the antipodals was in progress, a larger number of cases were noted in which there was no evidence of disintegration.

At 10 to 12 days after pollination ovules were observed in which the antipodals had disappeared, but the space the antipodal tissue had occupied often could be identified by the presence of indentations of irregular outline at the tip of the endosperm and strands of the partly digested nucellar tissue which formerly had surrounded the antipodals (pl. 2, *A*). A partial or complete disappearance or resorption of the antipodal tissue at this period was noted in somewhat less than half of the ovules examined. In the remainder, the surviving antipodals in some cases exhibited little or no evidence of renewed growth activity; in others, active growth was in progress. An example of the former is shown in plate 2, *B*, the antipodals here being organized in a small spherical group of cells. Similar groups in later stages of kernel development are shown in plate 2, *C* and *D*. The persistence of these essentially unaltered groups of antipodal cells in relatively advanced stages of kernel development may be interpreted as evidence that growth activity is not the determining factor in the survival or disappearance of the antipodals in the ontogeny of the caryopsis.

Initial stages of renewed growth activity of certain antipodal groups following the period of little or no growth immediately after fertilization were noted in young caryopses examined from 8 to 12 days after pollination. These early growth stages were characterized by an increase in the number and size of the cells without any apparent morphological differentiation within the antipodal tissue (pl. 3, *A*). In later developmental stages larger antipodal groups were noted in which there was a well-defined epidermis, a fairly definite arrangement of the subepidermal cells in radial rows, and within the innermost cells an accumulation of starch about the nuclei (pl. 3, *B*). With respect to the differentiation of an epidermis and the deposition of starch the antipodal tissue closely resembled the adjacent endosperm tissue in these stages; but with respect to cell size and nuclear size there was a noticeable difference, the cells and nuclei of the antipodal tissue being in general smaller than those of comparable regions in the endosperm. Presumably this size difference was due to the monoploid chromosome
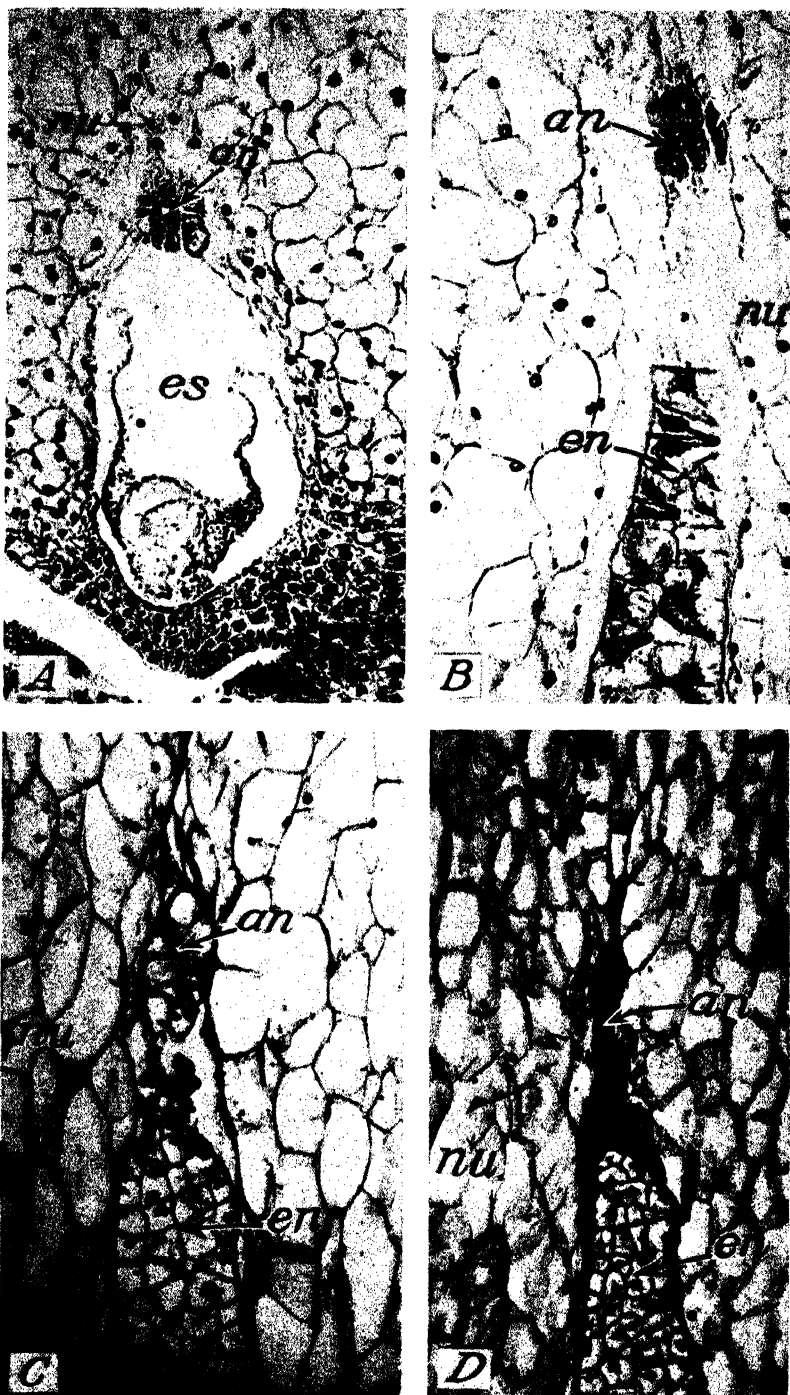
Antipodal tissue in early stages of kernel development; radial longitudinal sections: *A*, At time of fertilization; *B*, 4 days after pollination; *C* and *D*, 6 and 8 days after pollination, showing partial and advanced disintegration of antipodals. *an*, Antipodal tissue; *en*, endosperm; *es*, embryo sac; *nu*, nucellus. *A* and *B*, × 250; *C* and *D*, × 120.
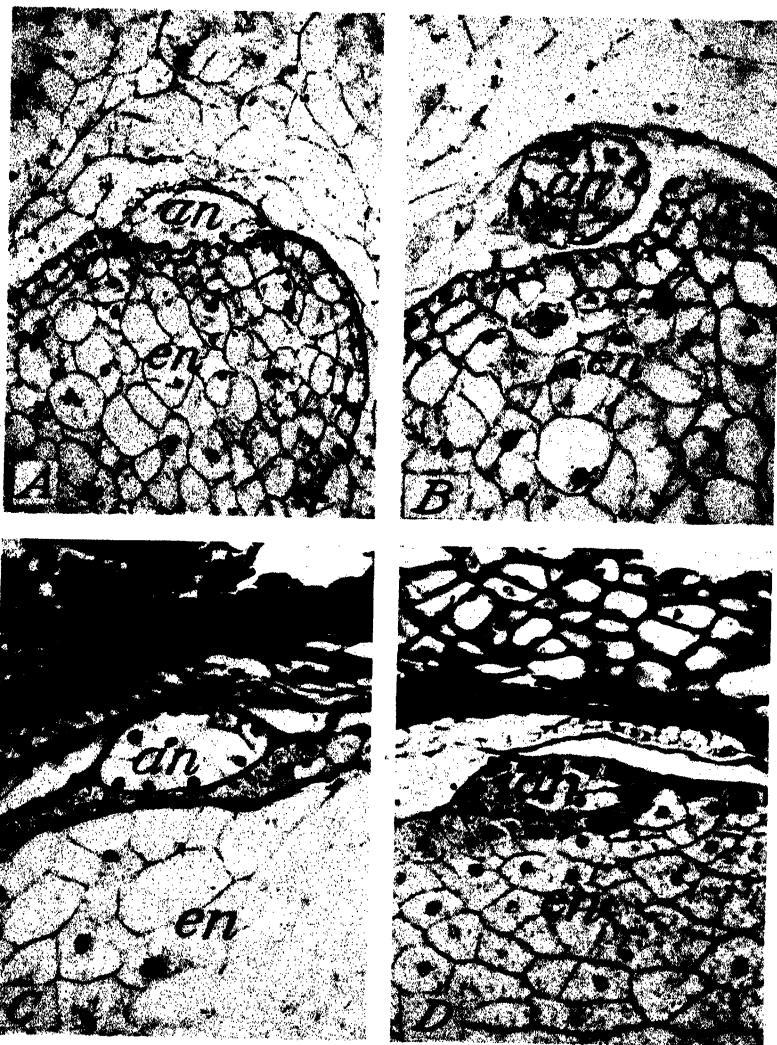
Antipodal tissue: *A*, Disintegration essentially completed, 11 days after pollination; *B–D*, persistence of antipodals as small groups of undifferentiated cells, 12, 23, and 26 days after pollination. *an*, Antipodal tissue; *en*, endosperm. *A*, × 120; *B–D*, × 200.

PLATE 3
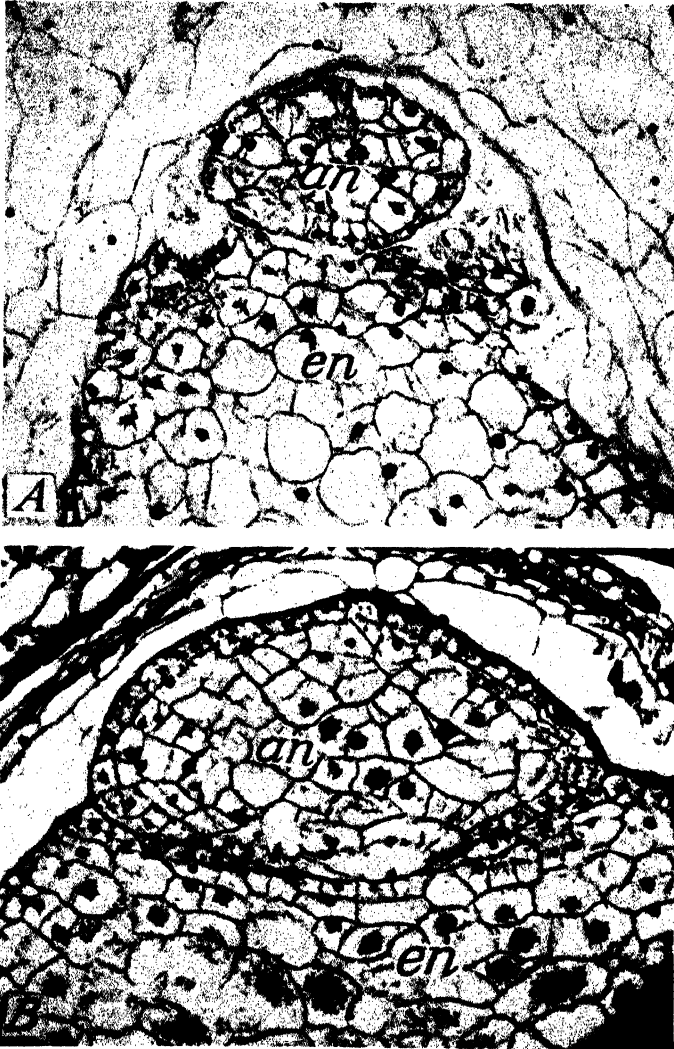
Actively developing antipodal groups: *A*, At 11 days after pollination, with as yet no apparent orderly arrangement or differentiation of the antipodal tissue; *B*, at 22 days after pollination, showing a well-defined epidermis and evidence of internal differentiation. *an*, Antipodal tissue; *en*, endosperm. × 160.
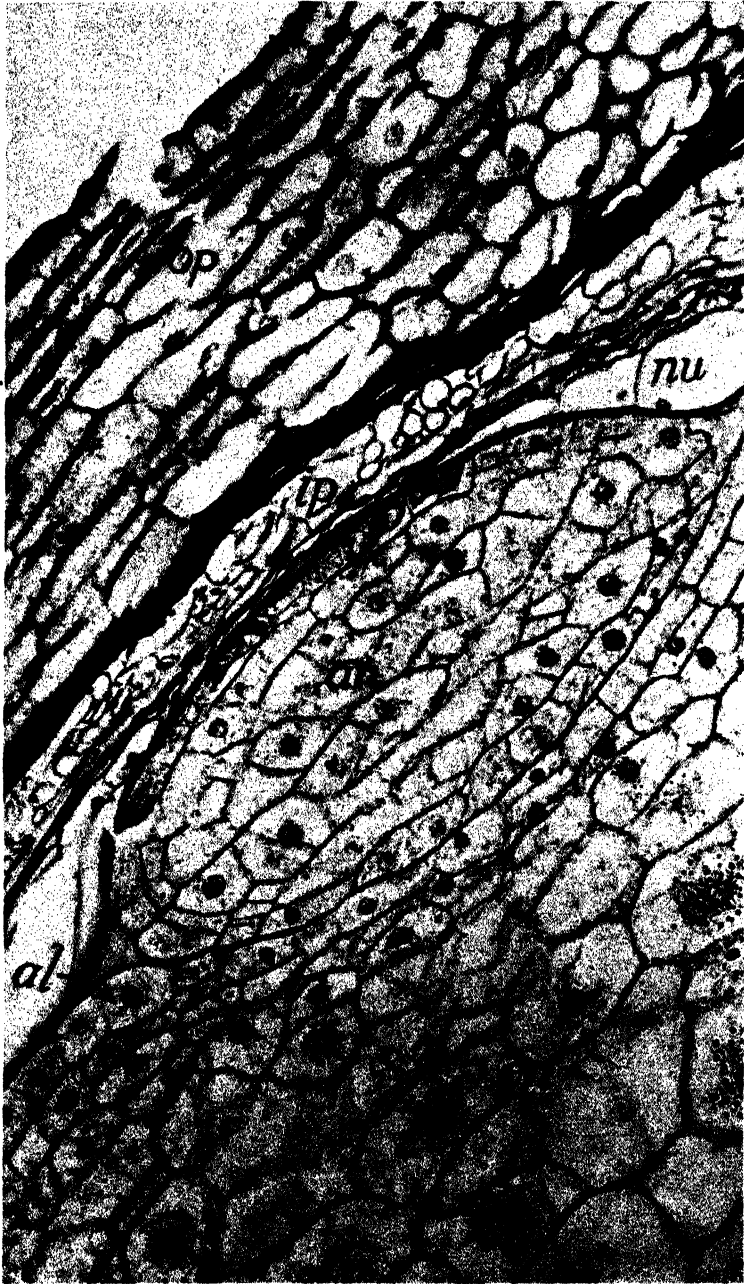
An antipodal group and surrounding tissues of a young caryopsis 20 days after pollination, highly magnified to show cellular differentiation. Epidermis of the antipodal tissue and the epidermis or aleurone layer of the endosperm sharply delimited: *an*, Antipodal tissue; *en*, endosperm; *al*, aleurone; *nu*, nucellus; *ip*, inner pericarp; *op*, outer pericarp. × 300.

constitution of the antipodal tissue and the triploid constitution of the endosperm, since there is ordinarily a definite correlation between nuclear and cell size and number of chromosome sets.

Antipodal groups in intermediate stages of development were noted in which no reserve starch was present, although starch grains were present in abundance in the adjacent endosperm tissue (pl. 4). These groups not infrequently were as fully differentiated as were those of the same age in which starch was being deposited.

The antipodal tissue occupies a position in the crown region of the kernel during the more advanced stages of kernel development (pl. 5, *A*). During early ontogenetic development there was a progressive movement of the antipodals away from the micropylar region of the nucellus, where they were located in the initial stages of kernel development, toward the apical or crown region, this movement taking place as the endosperm enlarged and expanded into the region formerly occupied by the nucellus. During this translocation the antipodals retained the same relative position with respect to the endosperm, i. e., they remained at or near the crest of the expanding region farthest removed from the embryo.

At the 16-day stage of development (pl. 5, *A*) a very limited portion of the nucellus remained between the antipodal tissue and the ovary wall or pericarp. Integument tissue was visible only at the stylar-canal region, and cellular disintegration had commenced in the internal region of the pericarp in the subcrown region on both the abgerminal and the germinal face of the caryopsis. At the 20-day stage (pl. 4) the antipodal group was in direct contact with the inner pericarp except for an intervening noncellular remnant of the nucellus. The outer and inner regions of the pericarp were separated by a crushed layer of disintegrating cells.

Although the nucellus disintegrates almost completely and the pericarp is compressed and the constituent cells are much flattened as maturity approaches, there was no evidence of a similar compression or disintegration of the antipodals during the same stages.

Growth of the antipodal tissue in many instances persisted until the caryopsis approached morphological maturity. A large, essentially mature antipodal group, consisting of hundreds of cells closely packed with starch and completely surrounded by a fully differentiated epidermis without reserve starch, is shown in plate 5, *B*. The relative positions of the antipodal tissue, the endosperm, and the pericarp are shown in plate 5, *B*. This antipodal group, as well as the group shown in plate 4, was located in the subcrown region of the abgerminal face of the caryopsis rather than directly beneath the silk-attachment region at the crown of the kernel as in plate 5, *A* (cf. pl. 11, *B*). A more highly magnified portion of the antipodal group shown in plate 5, *B*, is reproduced at a slightly different focus in plate 5, *C*. This figure demonstrates the marked similarity in the cellular organization of the antipodal tissue and the tissue of the adjoining endosperm. Noteworthy is the marked resemblance between the epidermis of the antipodal tissue and the epidermis or aleurone layer of the endosperm. Neither of these epidermal layers contained starch grains, although the adjacent cells in both the endosperm and the antipodal tissue were filled with starch grains. Whether aleurone grains were present in the epidermis of the antipodal tissue as well as in the aleurone layer of the endosperm was not determined.

INTEGUMENTS

In many members of the grass family, especially the cereal grains, integument tissue persists in the mature caryopsis and forms a definite layer, the seed coat. Ordinarily it is the inner integument that persists, the outer integument disappearing as the kernel matures. There is no seed coat in the maize caryopsis, according to the observations of Guérin (*15*), Poindexter (*33*), Haddad (*19*), and others who have studied the developmental morphology of the maize caryopsis. Haddad observed a complete resorption of the integumental tissues in the hybrid between two inbred strains of sweet corn within 20 days after pollination, and a similar resorption occurred somewhat later in the parental inbred strains. However, True (*47*) and Weatherwax (*52*) stated that crushed remnants of integument tissue were present at maturity, and Weatherwax referred to this remnant as a testa. These conflicting reports suggest that different varieties of maize may differ appreciably in the extent to which the integuments are resorbed. But in no case has a typical seed coat consisting of a well-defined layer of integument tissue been reported in maize.

There is at present a conspicuous lack of uniformity in the use of the term "seed coat" as applied to the caryopsis of maize and other grasses. The pericarp has been called the seed coat by Weatherwax, and Artschwager has referred to the pericarp plus the integuments as the seed coat. Others have referred to the persistent nucellar and integument tissue as the seed coat. In physiological researches on the fruits of various members of the grass family, especially researches concerned with permeability, the terms "seed coat", "testa", and "seed coat membrane" often have been applied indiscriminately to the various portions of the external covering of the caryopsis, irrespective of their true morphological nature. According to long-established usage the term "seed coat" is properly applied only to the structure or structures present in the mature caryopsis which originated solely from integument tissue. When separate layers are formed by the two integuments the hard outer layer is called appropriately the "testa." As there seems to be no plausible reason for changing these definitions, the long-established usage of these terms is here retained. A careful developmental study of the integuments was made in order to determine whether or not a seed coat was present in the strains of maize under investigation.

The integuments of the mature ovule in *Zea* consist of thin-walled parenchyma cells. The inner integument is typically two cell layers in thickness except in the region of the micropyle, where there are from three to five layers. The outer integument has typically either two or three cell layers, but in the integument-insertion region and at the stylar-canal region there are from three to five or more layers of cells. Active growth of the integuments to keep pace with the increase in

EXPLANATORY LEGEND FOR PLATE 5

Antipodal tissue in kernels at various stages of maturity: *A*, Crown region of an immature caryopsis 16 days after pollination in radial longitudinal section at right angles to the germinal face, showing relative position of antipodal tissue. *B*, A morphologically mature antipodal group 43 days after pollination, packed with starch and surrounded by a well-defined epidermis closely resembling the aleurone layer or epidermis of the adjoining endosperm. Reproduced at a relatively low magnification to show the relative positions of the antipodal tissue, the endosperm, and the pericarp in the region of the caryopsis occupied by the antipodals. *C*, A portion of *B* more highly magnified to show cellular details more clearly. *an*, Antipodals; *en*, endosperm; *al*, aleurone; *nu*, nucellus; *ii*, inner integument; *oi*, outer integument; *p*, pericarp; *sc*, stylar canal; *sa*, silk attachment region. *A*, × 25; *B*, × 80; *C*, × 200.
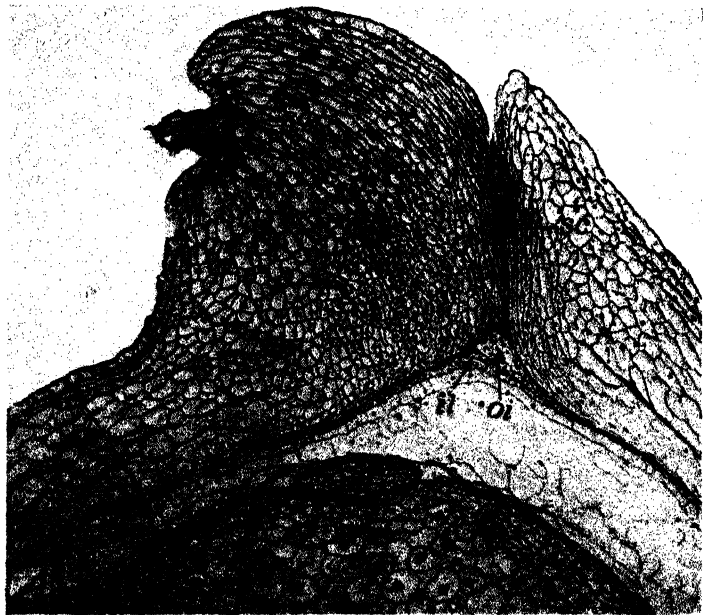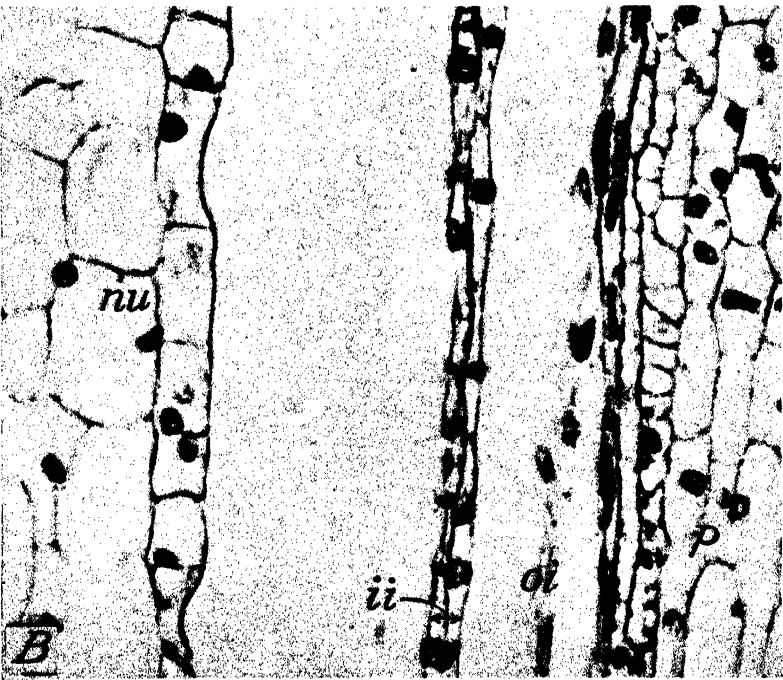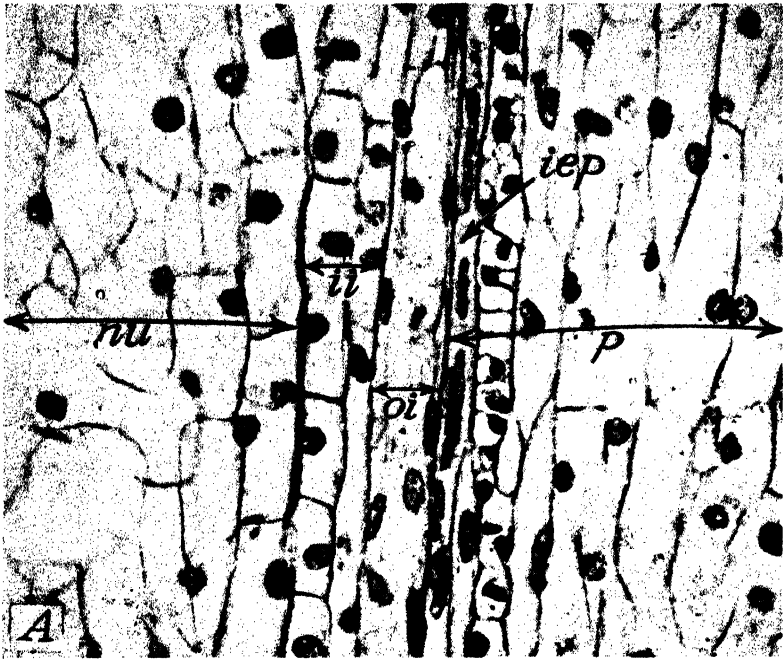
Integuments in the abgerminal region of a young caryopsis 7 days after pollination: *A*, Near the chalaza; *B*, In the subcrown region. Disintegration well advanced in outer but not in inner integument. Cell walls disappear first; later the cytoplasm and nuclei are resorbed. *ii*, Inner integument; *oi*, outer integument; *nu*, nucellus; *p*, pericarp; *iep*, inner epidermis of the pericarp. × 600.

the size of the ovule was in progress at the time of pollination and during the early embryonic stages. Cell-division figures were observed, especially in the basal regions of the integuments resulting in anticlinal wall formation. In later developmental stages, after disintegration of the integuments had begun, nuclear divisions continued even in regions where the cell walls had almost completely disappeared.

Initial stages in the disintegration of the integuments were noted in certain definitely localized regions in samples examined 3 days after pollination. These regions involved the inner integument over the germinal face of the young caryopsis and the outer integument over the abgerminal face at the chalazal region and in the subcrown region between the stylar canal and the base of the ovule. In 7-day samples (pl. 6, *B*) the break-down of the integuments in these regions was well advanced and was spreading rapidly into the other parts of the integuments. The illustrations of the integuments in plates 6 and 7 are photomicrographs of median radial longitudinal sections cut at right angles to the germinal face of the kernel.

The order of disappearance of the cell constituents of the integuments followed a regular sequence. First the walls of the cells disappeared (pl. 6, *A*). Then the cytoplasm became much reduced in amount (*oi*, pl. 6, *B*). Finally the nuclei of the cells associated with scattered remnants of cytoplasm were all that remained (pl. 7, *B*), and these subsequently disappeared more or less completely. The cell nuclei often remained for many days after the cell walls had entirely disappeared.

Although the inner integument began to disintegrate very early over the germinal face of the kernel, a similar disintegration of the inner integument over the abgerminal face did not take place until the outer integument in this region was in an advanced stage of disintegration (pl. 6, *B*). In the outer integument the break-down of the tissue occurred simultaneously in both the inner and outer cell layers (pl. 6), but the outer of the two cell layers of the inner integument regularly disappeared in advance of the inner layer of cells (pl. 7, *A*).

The inner integument was in an advanced stage of disintegration over the germinal face of the kernel 35 days after pollination. Occasional isolated nuclei were present between the inner epidermis of the pericarp and the outer wall of the nucellar epidermis (pl. 7, *C*), but elsewhere between these two layers at this same stage of kernel development, as illustrated in the lower part of this same figure (*C*), (*D*) there was no remaining trace of the inner integument tissue.

A complete disappearance of both integuments over most of the abgerminal face of the nearly mature kernels was the typical condition in specimens examined approximately 40 days after pollination (pl. 7, *D*). In the particular region of the kernel from which this photograph was taken there was no trace of any remnant either of integument tissue between the suberized nucellar membrane and the pericarp or of nucellar tissue between the aleurone and the nucellar membrane. However, in other regions of this preparation and in other specimens of the same and later stages of maturity an integument remnant in the form of flattened nuclei embedded in strands of cytoplasm occasionally could be identified. But no appreciable amount of

integument tissue in the form of a continuous covering about the kernel was present in the mature caryopsis.

On the basis of this study of the history of the integuments during kernel development, a study which involved the examination of a very complete ontogenetic series of stages from the commercial variety Pride of Michigan, as well as a survey of a limited number of other stocks of maize, it was concluded that the maize caryopsis has no true seed coat. No appreciable amount of integument tissue was present at maturity. Scattered remnants representing the cell contents of integument tissue which had not been completely resorbed were noted in certain regions of some samples, but this remnant did not comprise a continuous layer or covering which properly could be referred to as a seed coat. In the mature caryopsis the suberized nucellar membrane was the only structure of constant morphological value present between the aleurone layer and the pericarp.

NUCELLAR MEMBRANE

During the intermediate period in the development of the kernel, the nucellar membrane of the mature caryopsis differentiates as a suberized thickening of the outer cell walls of the epidermis of the nucellus. At the time of fertilization and during the initial stages in kernel development, the cells comprising this epidermal tissue had relatively thin walls, and tests with Sudan III and Sudan IV indicated that the walls were not suberized in these early stages. During the period from 6 to 10 days after pollination, the outer walls of the cells of the nucellar epidermis increased in thickness and became suberized (pls. 6, B; 7, A), first in the region adjacent to the chalaza (pl. 8, A, B) and later over the entire epidermis. The photomicrographs showing the development of the nucellar membrane were obtained from median longitudinal sections cut at right angles to the germinal face of the kernel. At 16 to 18 days following pollination, a definite suberized layer appreciably thicker than the original wall was present as an integral part of the nucellar epidermis (pl. 8, C, D). Soon thereafter the developing endosperm, which had already digested the inner region of the nucellus, encroached upon and destroyed the cellular organization of the outer portion of the nucellus, including the epidermis; only the suberized outer wall was left intact (pl. 8, E, F).

As these changes are taking place in the nucellus, the integuments are being resorbed. As stated previously, the cell walls of the integument tissue disappear first, the nuclei and cytoplasm of the cells often persisting for some time after the walls of the cells have vanished. Disintegration of both integuments is well advanced before the nucellar epidermis becomes extensively suberized (pl. 8, C, D). This relatively early and virtually complete disappearance of the integuments in maize is strikingly different from the situation in many other grasses in which integument tissue persists and forms the seed coat of the mature caryopsis.

The nucellus is represented in the mature caryopsis only by this suberized membrane and by discontinuous noncellular remnants of nucellar tissue between this layer and the aleurone (pl. 9). As previously stated, no true seed coat derived from integument tissue was observed in the mature caryopsis. The suberized nucellar membrane and the pericarp constituted the sole coverings of the maize kernel.

Stages in the disappearance of the integuments in germinal (*A–C*) and abgerminal (*D*) regions of immature caryopses: *A*, 7 days; *B*, 18 days; *C*, 35 days; and *D*, 40 days after pollination. In *A* the cell walls have disappeared in the outer of the two cell layers of the inner integument, the inner layer of cells being essentially intact. In *B* the integument remnant consists of disintegrating free nuclei and traces of cytoplasm lying between the inner epidermis of the pericarp and the nucellar epidermis. *C* shows a similar but more discontinuous remnant at a later stage. In *D* no integument tissue remains, consequently the nucellar membrane is directly in contact with the pericarp. *al*, Aleurone; *en*, endosperm; *iep*, inner epidermis of the pericarp; *ii*, inner integument; *ir*, integument remnant; *nm*, nucellar membrane; *nu*, nucellus; *nue*, nucellar epidermis; *p*, pericarp. × 600.
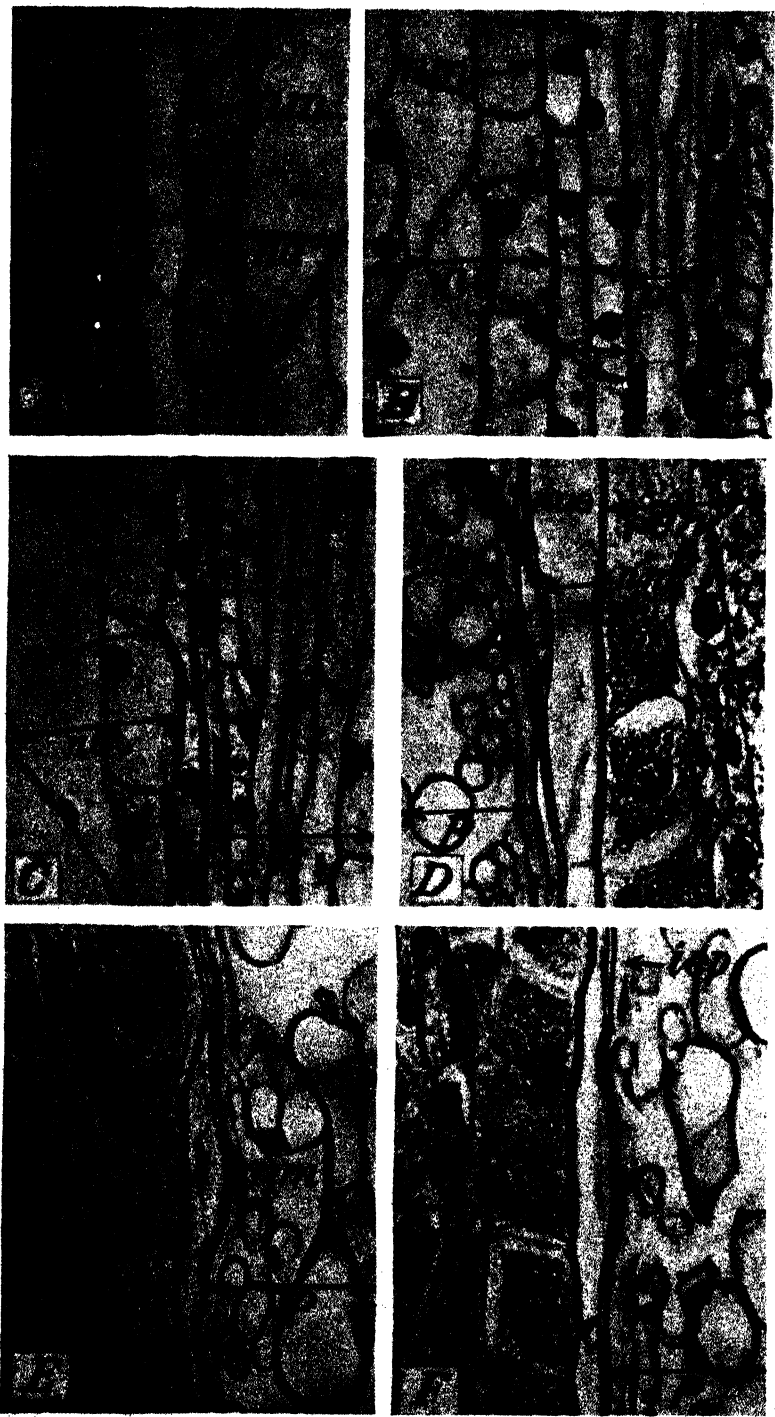
The suberized nucellar membrane probably was observed by at least two of the early investigators of kernel morphology in maize. True (*47, p. 220*) mentioned the presence of a conspicuous "cuticle" associated with the nucellar epidermis of the ripe grain in *Zea*. Guérin (*15, p. 7*), after describing the disappearance of the integuments in maize during ontogenetic development, stated that at maturity "L'épiderme du nucelle a de même complètement disparu"; but he also referred to a seed coat represented only by "une bande comprimée dans laquelle il est impossible de retrouver la moindre structure cellulaire."

Brown (*5, 6*) was the first to demonstrate the semipermeable nature of the seed coat in a member of the grass family. Later, in certain cereals and other members of the Gramineae, Harrington and Crocker (*20*), Andersen (*1*), Gurewitsch (*17*), Tharp,[16] and Krauss *(24)* described a suberized semipermeable membrane associated with the integuments rather than with the nucellus, as in *Zea*. In *Zea*, the physiological researches of Shull and Shull (*40*), Orton (*32*), Beeskow,[17] and Tharp [16] demonstrated that the protective covering of the embryo and endosperm exhibits semipermeable properties. Beeskow attributed these properties to a layer, chiefly fatty or lipoid in character, formed by the disintegrating integuments. But it is more probable that the semipermeability of the maize caryopsis is due primarily to the presence of a suberized membrane of nucellar origin, an interpretation that has already been applied to the related forms having similar membranes, derived apparently from integument tissue.

PERICARP

The transformation of the ovary wall into the pericarp in *Zea* is a gradual process and requires the entire period from fertilization to maturity for its completion. A progressive series of changes is involved, which includes growth by cell division and cell enlargement, disintegration and collapse of cells in certain regions, extensive lignification of cell walls, and a final compression of the entire tissue into the relatively thin protective covering of the mature caryopsis. The amount of growth involved is very appreciable, since the dimensions of the mature kernel are from 8 to 12 times as great as those of the mature ovary from which it is derived. The following account of the development of the pericarp is essentially in agreement with the previous researches of True (*47*), Guérin (*15*), Randolph (*34*), and Haddad (*19*), which have provided a fairly complete description of the essential features of the process.

The ovary wall, which differentiates into the pericarp as kernel development proceeds, consists chiefly of thin-walled, undifferentiated

[16] THARP, W. H., JR.   See footnote 6.
[17] BEESKOW, H. C.   See footnote 5.

parenchyma cells at the time of fertilization. There were many cell-division figures in the ovary-wall tissue, especially near the base of the ovary, in the initial stages of kernel development. Adjoining the inner region of the ovary wall, numerous small, isodiametric cells were being formed by a subdivision of the larger cells in this region. This growth activity began soon after fertilization and continued for 10 to 14 days (pl. 10). Elsewhere scattered division figures also were noted less frequently. These observations on early cell-division activity in the differentiating pericarp are not in agreement with the statement of Haddad (*19*) that the increase in the size of the ovary wall following the 1-day stage was accomplished by an increase in cell size rather than by an increase in the number of cells. An increase both in the number and in the size of the cells apparently contributed to the growth of the pericarp tissue.

Within 10 days after pollination the ovary wall, or pericarp, had increased to approximately three times its original length and twice its original diameter. This increase was accomplished chiefly by an enlargement of the cells in the outer region and by both cell division and cell enlargement in the inner region (pl. 10). At about the 10-day stage the cells that were approximately halfway between the outer and inner limits of the pericarp in the subcrown region surrounding the base of the style began to disintegrate (pl. 11, *A*) and later became crushed together, forming a thin layer of noncellular material (pl. 11, *B*). At 10 to 14 days the inner epidermis consisted of cells which in the radial longitudinal sections appeared very much elongated and narrow (cf. pls. 7, *D*, and 8, *B*); in transverse sections they were small and nearly circular in outline. There were frequently one to three or more additional layers of cells of this type adjoining the inner epidermal layer. Just beneath these cells there were a few layers of cells rather similar to the epidermal cells but with their long axes at right angles to those of the epidermis; thus, these deeper lying cells appeared as elongated cells in the cross sections and as small circular cells in the longitudinal sections (pl. 7, *B*).

The pericarp increased in thickness up to 9 to 12 days in the crown region and up to 15 to 18 days in the basal region. This difference in the time at which different parts of the pericarp attained a maximum thickness was due primarily to the fact that the disintegration of the middle portion of the pericarp took place at the crown region earlier than in the basal region. Thereafter a cessation of lateral growth activity, accompanied by disintegration in the middle region and compression of the tissue laterally, resulted in a gradual decrease in the thickness of the pericarp in the later stages of maturity. However, elongation of the pericarp from the base of the kernel to the crown region and increase in circumference continued until the kernel was morphologically mature and the embryo and endosperm ceased to expand.

The disintegration and partial resorption of the cells in the central portion of the subcrown region of the pericarp (pl. 11, *A*) were followed by a collapse and compression of the tissue in this region, leaving a layer of noncellular material separating the outer and inner regions of the pericarp (*B*). This partial disintegration and collapse of cells in the middle region of the pericarp, which began first in the subcrown region, gradually extended toward the base of the kernel and thus delimited the inner and outer pericarp over the entire surface of

Sections from the abgerminal face of nearly mature kernels in the region of the nucellar membrane: *A*, 40 days after pollination; *B*, 48 days after pollination. In *A* the nucellar membrane is in contact with the inner epidermis of the pericarp, but there is a narrow space between the membrane and the aleurone which is partly filled with disorganized nucellar tissue. In *B* the nucellar membrane appears as a narrow band of uniform width between the aleurone layer and the inner margin of the pericarp. *nm*, Nucellar membrane; *al*, aleurone layer; *p*, pericarp. × 600.
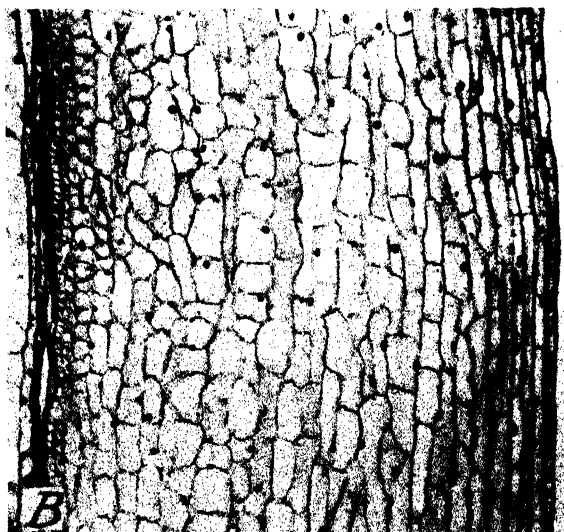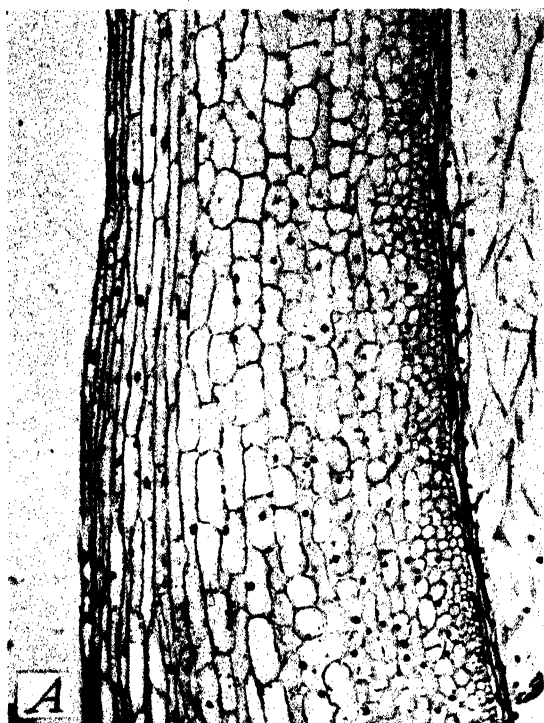
Median longitudinal sections of the pericarp of young kernels 10 days after pollination, the sections being taken at right angles to the germinal face of the kernel: *A*, Germinal face; *B*, abgerminal face midway between the tip and the base of the kernel. × 125.
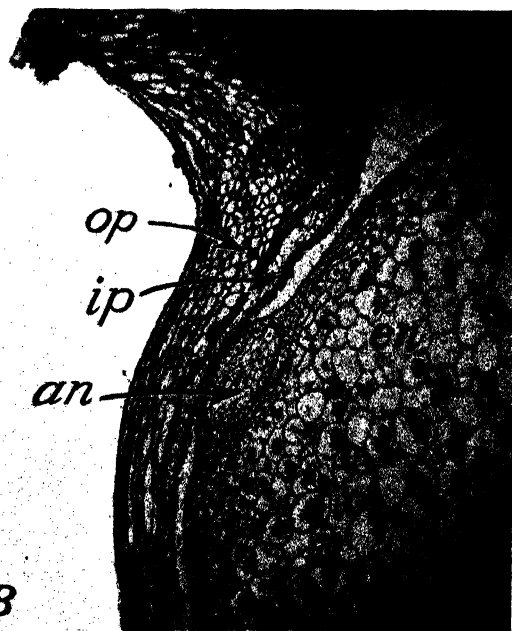
The pericarp in the subcrown region over the germinal face of developing kernels: *A*, Highly magnified to show clearly the initial stages of cellular disintegration in the middle region of the pericarp 10 days after pollination; *B*, a lower magnification showing a more extensive portion of the subcrown region 20 days after pollination. *op*, Outer pericarp; *ip*, inner pericarp; *an*, antipodal tissue; *nu*, nucellus; *en*, endosperm. *A*, × 160; *B*. × 60.
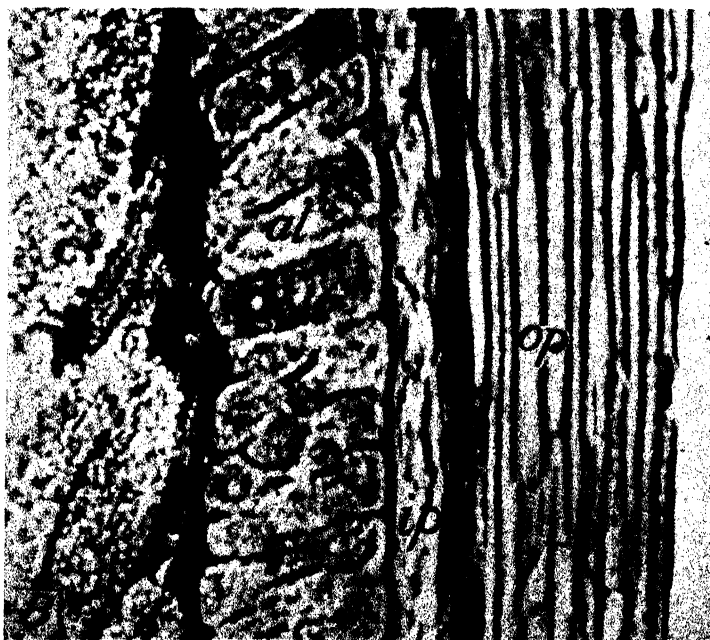
Longitudinal sections of pericarp and adjacent tissues in midabgerminal region of developing kernels: *A*, 23 days after pollination; *B*, 40 days after pollination. *ip*, Inner pericarp; *op*, outer pericarp; *nu*, nucellus; *al*, aleurone; *en*, endosperm. × 250.
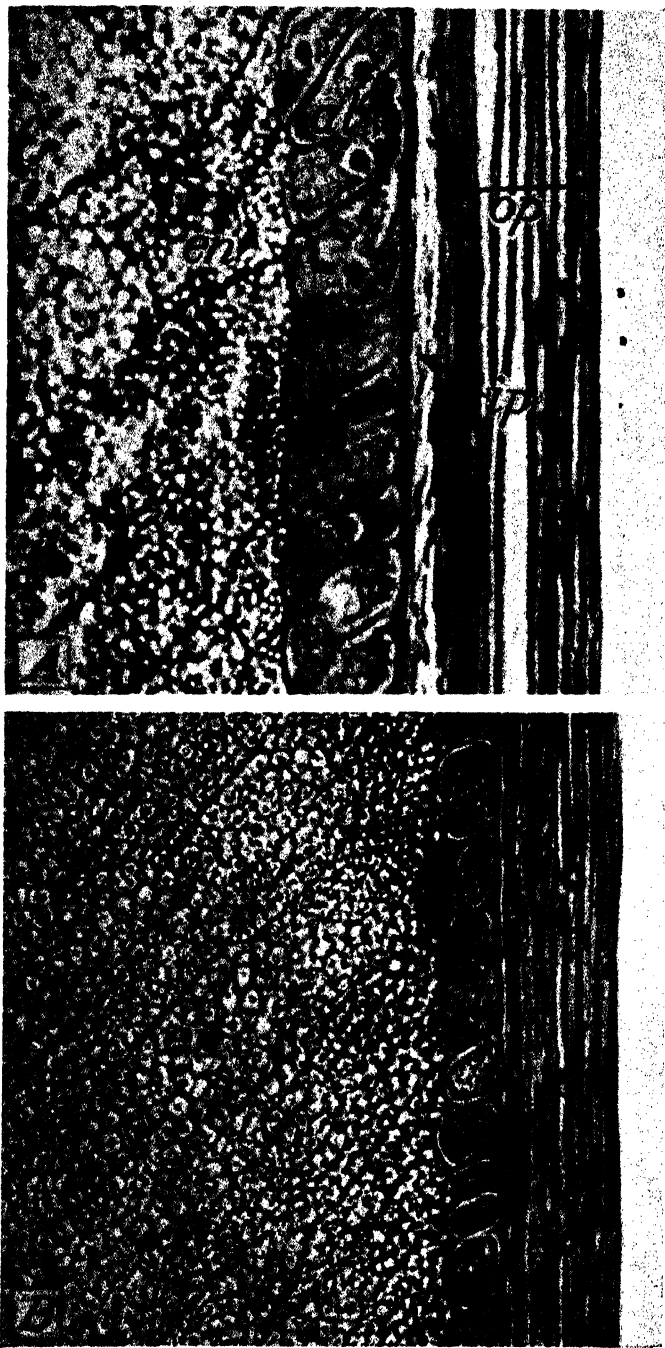
Longitudinal sections of pericarp and adjacent tissues in midabgerminal region of nearly mature (*A*) and mature (*B*) kernels: *ip*, Inner pericarp; *op*, outer pericarp; *p*, pericarp; *al*, aleurone; *en*, endosperm. *A*, × 250; *B*, × 160.

the kernel, except in the silk-attachment region and in the basal attachment region of the kernel. The basipetal succession of this process of tissue differentiation was in general characteristic of the order of development in the pericarp throughout its ontogenetic history.

The cells of the outer pericarp increased materially in size and gradually developed relatively thick walls with numerous simple pits during the period from 20 to 40 days after pollination (pl. 12). Intercellular spaces were lacking in the outer region; the cells remained intact and did not become separated from one another as development proceeded. A strikingly different type of behavior characterized the cells of the inner pericarp during this period. Here the cells remained thin-walled and, failing to increase in size as the expansion of the pericarp progressed, they became widely separated from one another (pl. 12, *A*). During the same period, however, the cells of the inner epidermis of the pericarp elongated very appreciably and to a sufficient extent to maintain the continuity of the epidermis as a well-defined layer of cells (pl. 7, *B*, *C*). Thus the epidermal cells were long and narrow viewed in longitudinal sections at right angles to the germinal face of the kernel, but in cross sections they were nearly circular. Eventually, as the kernel continued to enlarge in circumference these inner epidermal cells were drawn apart laterally and presented the appearance of a network, the so-called "cellules tubulaires" of Guérin, over the inner face of the pericarp.

The final stages of pericarp development were characterized by a continued increase in the thickness of the cell walls, especially the tangential walls, of the outer pericarp and by a gradual compression laterally of the entire pericarp (pls. 12, 13). Plate 12, *A* and *B*, and plate 13, *A*, may be compared directly, since they are reproduced at the same magnification, but plate 13, *B*, is reproduced at a somewhat lower magnification. As maturity approached the inner pericarp became more and more compressed until it was scarcely distinguishable as a separate region (pl. 12, *A* and *B*), except near the base of the kernel where it remained separate. The lumina of the cells in the outer pericarp gradually disappeared, owing to lateral compression and increase in wall thickness. In the mature pericarp (pl. 13, *B*) the much thickened and extensively lignified tangential walls of the individual cells were closely appressed and formed a firm protective covering for the caryopsis.

SUMMARY AND CONCLUSIONS

The morphology of the pistillate spikelet, the ovary, and the ovule in maize (*Zea mays* L.), is described briefly, and this description is followed by a detailed account of the developmental history of the caryopsis, together with a consideration of the time factor, temperature, and seasonal variations in relation to pollen-tube growth, fertilization, and kernel development.

Pollination is followed by germination of the pollen and establishment of the pollen tube in the stigmatic hairs of the silk within 5 to 10 minutes under ordinary conditions of temperature and humidity. Active protoplasmic streaming accompanies the early growth stages of the pollen tube. During the period from 2 to 4 hours after pollination the male nuclei migrate from the pollen grain into the pollen tube

through the actively streaming protoplasm and begin their passage to the embryo sac.

The time interval from pollination to fertilization under field conditions in which the maximum day temperatures ranged from 25° to 30° C. and the minimum night temperatures ranged from 13° to 17° was approximately 16 hours for silk lengths of 3 to 5 cm and 23 hours for silk lengths of 15 to 18 cm.

The primary endosperm nucleus divides within 2 to 4 hours after fertilization, and four to eight endosperm nuclei are formed at the time the zygote divides to form the two-celled proembryo at 10 to 12 hours after fertilization.

The major morphological changes in the transformation of the pistil into the mature caryopsis involve (1) the complete displacement of the ovule and integument tissues by the embryo and endosperm, (2) the formation of a suberized membrane derived from the epidermis of the nucellus, and (3) the transformation of the ovary wall into the pericarp. These changes were traced in a close series of stages obtained from material fixed at definite intervals following controlled pollinations.

The proembryo develops very irregularly in the initial stages, with no very orderly arrangement of cells or sequence of cell divisions. It conforms with the classical descriptions of grass embryos only to the extent that regular tiers of three or four cells with parallel cross walls occasionally are formed; but this type of proembryo is only one of a diverse series, the members of which differ markedly in the manner in which wall formation takes place.

The sectors of the proembryo in the initial stages of development are rapidly obscured as growth continues, being virtually obliterated well in advance of the delimitation of the plumule-radicle axis of the mature embryo. This early disappearance of the first cleavage planes and the variation in their relative positions in different embryos make it impossible to determine or to predict with reasonable accuracy which sector or sectors give rise to the root and shoot regions of the plumule-radicle axis.

The epidermis is differentiated as a separate tissue, first over the apical region of the proembryo and somewhat later elsewhere, during the period from 7 to 10 days after pollination.

The axis of the more mature embryo may be recognized 9 or 10 days after pollination as a group of meristematic cells in the anterior portion of the embryo. This permanent plumule-radicle axis is a lateral structure oriented in an oblique position with reference to the axis of the proembryo. At maturity the plumule-radicle axis assumes a position essentially parallel to that of the scutellum, by which it is almost completely surrounded.

The major portion of the proembryo, which does not contribute to the main axis of the embryo proper, continues to enlarge and becomes the massive scutellum of the mature embryo.

The basal suspensor region of the proembryo ceases growth soon after the plumule-radicle axis forms and persists merely as a vestigial organ at the base of the more mature embryo.

The coleoptile arises as the first ridge of tissue which develops first above and later around the stem meristem. The first seedling leaf arises next as a second ridge of tissue identical with but opposite that which gives rise to the coleoptile. Later three to five additional seed-

ling leaves make their appearance, as the embryo matures, in positions alternating with those of the coleoptile and the first seedling leaf.

The primary seedling radicle is differentiated within the internal region of the proembryo above the suspensor; as the cells of this region multiply they assume orderly arrangements and thus delimit the epidermal tissue and underlying portions of the radicle primordium. The tissue of the proembryo surrounding the young radicle develops as an ensheathing structure, the coleorhiza.

The limited scope of the investigation precluded a consideration of the homologies of the scutellum, the coleoptile, the coleorhiza, and other organs of the embryo. •

The endosperm in its early development passes through the usual free-nucleate stage and becomes cellular approximately 4 days after pollination. At first, cell-division activity is prevalent throughout the endosperm; later it becomes localized in the peripheral region with periclinal wall formation taking place repeatedly in the epidermal layer and somewhat less frequently in the subepidermal cells.

The epidermal layer of the endosperm ceases active periclinal wall formation approximately 20 to 22 days after pollination and subsequently becomes the aleurone layer. Cell-division activity persists in the subepidermal cells, particularly in the middle region between the base and the tip of the endosperm, until maturity is approached. The growth of the inner portion of the endosperm during most of the developmental period is by cell enlargement rather than by active cell division.

The antipodal cells often proliferate during kernel development and persist in the mature caryopsis as an oval-shaped mass of haploid tissue between the aleurone layer and the pericarp at the tip of the kernel.

The integuments of the ovule disintegrate and virtually disappear during the early development of the caryopsis. Scattered noncellular remnants of integument tissue in the form of a very thin discontinuous layer were occasionally noted in advanced stages of kernel development. A seed coat consisting of a well-defined layer of integument tissue is lacking in the varieties of maize examined during the course of this investigation.

A suberized nucellar membrane derived from the outer wall of the nucellar epidermis was present in the mature caryopsis as a continuous, well-defined layer between the aleurone and the pericarp.

The ovary wall is transformed into the pericarp of the kernel. The process involves cell multiplication and cell enlargement in the early stages, subsequent disintegration and collapse of the middle region, and an extensive thickening of the tangential walls of the cells in the outer region, which is followed by a compression of the entire tissue to form the tough protective covering of the mature caryopsis.

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RESISTANCE TO BACTERIAL WILT OF OPEN-POLLI-NATED VARIETIES OF SWEET, DENT, AND FLINT CORN [1]

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INTRODUCTION

Studies have been made on resistance of approximately 120 open-pollinated varieties of sweet, dent, and flint corn (*Zea mays* L.) to bacterial wilt, caused by *Phytomonas stewarti* (Smith) Bergey et al.

The resistance of a number of open-pollinated varieties of sweet corn had been tested earlier through natural infection by Smith (*6*),[3] Thomas (*8*), Rand and Cash (*4*, *5*), and others, whose work has already been reviewed by Ivanoff and Riker (*2*). Stated briefly, their results showed that the later sweet corn varieties were as a rule more resistant than the earlier ones. So far no extensive reports have been published on the varietal resistance of dent and flint corn to bacterial wilt. The occasional inclusion of some of these corns in resistance trials has shown that the dent corn varieties appeared more resistant than the sweet and flint corn varieties.

Corn growers from the Midwestern and Eastern States, where the wilt disease has been particularly prevalent and where late field corns are ordinarily grown, have often observed that field corn varieties withstood the disease better than sweet corn varieties. The belief in the higher resistance qualities of field corn as such has prevailed among some corn breeders who have attempted to cross sweet corn with field corn in the hope that they might obtain an early sweet corn variety with the highly resistant qualities of field corn. The question whether the field corn varieties grown in the Middle West are highly resistant because of some type of resistance inherent in these varieties or because of their relatively great height and lateness arose soon after it was found that lateness and height were associated with the resistance of Golden Bantam sweet corn inbreds and crosses (*2*).

The purpose of these studies was threefold: (1) To evaluate each variety, particularly those of the sweet corn group, for resistance to the disease, with a view to aiding the corn grower in his selection of varieties for planting in localities where the disease is of economic importance; (2) to investigate correlations of resistance with other plant characters within each of the three varietal groups; and (3) to compare the resistance of the sweet corn varieties with that of dent and flint varieties. The results are presented without further repetitions because they seem to be entirely in accord with those on sweet corn conducted during three seasons (Ivanoff and Riker (*2*)).

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MATERIALS AND METHODS

The corn tested consisted of 92 open-pollinated sweet, 17 dent, and 11 flint corn varieties. Four dent corn hybrids were also included. The material was obtained from various sources, and was characterized by its diversity in respect to several important plant characters, particularly height and time of maturity. Thus among each of these three varietal types of corn there were some varieties which were very early, reaching edible ear stage in about 60 to 70 days, others which were medium early, and still others, such as Honey June, Leaming, and Kutias, which were very late. The difference in maturity between the earliest and latest corn was more than a month. The height of the various varieties ranged from 32 to 123 inches. The trials were conducted in northern Illinois during the summer of 1935.

The methods employed in these studies for planting, inoculating, and taking results were similar to those reported by Ivanoff and Riker (*2*) in the study of resistance to bacterial wilt of inbred strains and crosses of sweet corn. The more important of these methods, briefly stated, are as follows:

Each variety was represented by at least two rows of about 45 plants each. One of the rows was inoculated and the other left for control. Some varieties were replicated from 2 to 15 times.

The plants were artificially inoculated at the most susceptible stage by means of a plant inoculator which has been described by the writer (*1*). The method of artificial inoculation has been found not only to be adequate, but also to possess advantages over natural infection in disease-resistance studies.

A standardized suspension of five cultures of *Phytomonas stewarti* was used for the inoculations.

The height of the plants was measured at the time of maturity. The lateness of a corn variety was judged by the silking date, which was the date on which 75 percent of the corn plants came into silk. The records on height and lateness were taken on the uninoculated control rows.

The degree of resistance of each inoculated plant was estimated on the basis of degree of injury at the time of harvesting. Each plant was given one of the following five indexes of resistance: 0, 25, 50, 75, and 100. These indexes corresponded to five more or less distinct degrees of disease injury. A plant which at the time of record taking was found dead, with all of its parts dry and light brown in color on account of the disease, was very stunted, and had a very small ear or no ear at all, received an index of resistance of 0. A plant which appeared normal, like the control plants of the same strain (even though the bacteria were present within its tissue), received an index of resistance of 100. Between these two extreme degrees of reaction to the inoculations, there were plants which exhibited three other degrees of injury intermediate between the two just mentioned. These plants were given indexes of resistance of 25, 50, and 75. The index of resistance for an entire strain was estimated as the arithmetical mean of the indexes of all the plants of that strain. These indexes corresponded roughly to the percentage yield at the time of harvesting. In comparing the indexes of resistance of two varieties, a difference of a few points should not be considered significant. All corn was planted May 24.

EXPERIMENTAL RESULTS

Great differences in resistance were found among the varieties of each of the three varietal groups. Within each group there were some varieties which died from the disease sometime before harvesting for canning and produced no ears at all, while a few other varieties reached canning stage with very little loss in yield. The majority of the varieties, however, occupied a place between these two extreme limits. Alphabetical lists of all the sweet, dent, and flint corn varieties tested, with a measure of the maximum height, silking date, and index of resistance for each variety, are given in tables 1 and 2.

TABLE 1.—*Alphabetical list of open-pollinated sweet corn varieties inoculated with Phytomonas stewarti in 1935, with an indication of their maximum height, silking date, and index of resistance* [1]

Variety [2]	Color of kernel	Maximum height	Silking date	Index of resistance
		Inches		
Abbott and Cobb Early	White	78	Aug. 6	55
Alameda	do	68	Aug. 1	23
Alameda Sweet	do	67	do	27
Alpha	do	58	July 25	0
Aroostook	do	58	do	0
Banana Cream, Will's	do	81	July 29	11
Bantam Evergreen	Yellow	87	Aug. 8	56
Banting	do	32	July 22	0
Burden Wonder Bantam	do	70	July 31	25
Black Mexican	Black	71	Aug. 5	41
Burbank Bantam	Yellow	68	Aug. 4	30
Canada Gold	do	55	July 31	0
Carmel Golden	do	58	Aug. 2	8
Charlevois	do	64	Aug. 6	9
Colossal, Maule's	White	90	do	52
Columbia	do	55	Aug. 2	0
Country Gentleman	do	77	Aug. 14	74
Crosby	do	73	Aug. 4	7
Dorinny	Yellow	49	July 25	0
Earliest, Vaughan's	White	52	July 27	0
Early Columbia	do	69	Aug. 1	19
Early Dow	do	52	July 25	10
Early Evergreen	do	89	Aug. 12	69
Early Fortune	do	54	July 29	0
Early Golden Sweet	Yellow	41	do	0
Early June, Will's	White	46	do	0
Early Market	do	43	July 31	0
Early Minnesota	do	75	Aug. 8	39
Early Saskatchewan	do	51	July 29	5
Early Sunshine	Yellow	63	do	10
Early Surprise	White	52	Aug. 2	5
Early Sweet	do	71	do	14
Early White Cory	do	53	July 29	0
Early White Market	do	54	July 25	0
Early Yellow Sensation	Yellow	54	July 29	0
Extra Early Adams	White	80	Aug. 2	53
Extra Early Bantam, Harris'	Yellow	67	July 29	0
Extra Early Golden	do	47	Aug. 2	0
Extra Early Golden Bantam	do	66	July 31	5
First of All, Maule's	White	70	Aug. 2	45
Ford Early	do	60	do	15
Gold Coin	Yellow	74	Aug. 6	41
Golden Bantam	do	62	Aug. 2	15
Golden Cory	do	61	July 29	10
Golden Country Gentleman, Henderson's	do	69	Aug. 8	47
Golden Cream	do	65	Aug. 6	65
Golden Early Market	do	53	July 24	0
Golden Evergreen	do	92	Aug. 6	75
Golden Gem	do	50	July 25	0
Golden Gentleman	do	65	Aug. 10	42
Golden Giant	do	77	Aug. 2	46
Golden Giant, De Lue's	do	69	do	66
Golden Rod	do	80	do	40
Golden Sixty Day	do	53	July 25	25
Golden Sugar, Ford	do	78	Aug. 10	85
Golden Sunshine	do	65	July 29	23
Honey June	White	120	Aug. 18	95

[1] The garden varieties, or the Adams varieties, usually grown in the Southern States, have been included in these studies in the sweet corn group.
[2] The names of the varieties are those supplied by the cooperators and no grouping of synonyms has been made. For detailed descriptions of sweet corn varieties see Tapley, Enzie, and Van Eseltine (7).

TABLE 1.—*Alphabetical list of open-pollinated sweet corn varieties inoculated with Phytomonas stewarti in 1935, with an indication of their maximum height, silking date, and index of resistance*—Continued

Variety	Color of kernel	Maximum height	Silking date	Index of resistance
		Inches		
Howling Mob	White	76	Aug. 2	51
Imperial Mammoth	do	81	Aug. 8	64
Improved Golden Bantam, Gill's	Yellow	64	July 31	16
Kingcrost Canning Bantam	do	58	do	30
Kingcrost Golden Bantam	do	56	July 29	30
Lead All, Maule's	White	97		75
Long Island Beauty	do	96	Aug. 15	77
Mammoth Late	do	77	Aug. 14	73
Mammoth White Cory	do	38	Aug. 2	0
Maule XX	do	80	Aug. 6	60
Mayflower	do	69	Aug. 2	37
Money Maker	do	94	Aug. 12	85
Narrow Grained Evergreen	do	88	Aug. 15	70
Nectar	do	82	Aug. 12	70
Ne Plus Ultra	do	62	do	70
New Jersey Early White	do	73	Aug. 2	44
Nuetta	Yellow-red	60	July 27	0
Oregon Evergreen	White	80	Aug. 11	68
Pickaninny	Black	43	July 23	0
Portland Market	White	52	Aug. 2	0
Sixty-Day White	do	49	July 27	0
Spanish Gold	Yellow	55	do	0
Standard Bantam	do	59	Aug. 6	10
Stowell Evergreen	White	85	Aug. 12	69
Sunshine	Yellow	58	Aug. 1	8
Super Golden Sweet	do	83	Aug. 10	54
Surecropper Sugar	White	103	Aug. 16	90
Surprise	do	46	Aug. 2	0
Sweet Orange	Yellow	77	Aug. 8	32
Texas Evergreen	White	106	Aug. 16	75
Tucker Favorite	do	111	do	90
Whipple Early White	do	68	Aug. 6	32
Whipple Early Yellow	Yellow	76	Aug. 7	42
Whipple White	White	82	Aug. 2	70
Wood Southern Sweet Corn	Yellow	117	Aug. 16	80

TABLE 2.—*Alphabetical list of open-pollinated dent and flint corn varieties and crosses inoculated with Phytomonas stewarti, with a measure of their maximum height, silking date, and index of resistance*

Variety	Replications	Maximum iheght	Silking date	Index of resistance
Dent:	*Number*	*Inches*		
Extra Early Minnesota 13	2	76	Aug. 4	43
Extra Early Northwestern Dent	4	67	July 29	21
Extra Early Rustler White Dent	1	73	Aug. 8	47
Falconer Yellow Dent	1	68	July 31	19
Funk 176A	1	104	Aug. 16	85
Golden Glow	2	98	Aug. 12	73
Golden King Yellow Dent	3	95	Aug. 7	70
Iowa hybrid 931	1	107	Aug. 8	85
Iowa hybrid 939	1	108	do	85
Iowa hybrid 942	1	108	Aug. 10	85
Krug	1	123	Aug. 14	80
Leaming	3	113	do	80
Minnesota 13	2	94	Aug. 7	75
Murdock	3	102	Aug. 11	78
Northwestern Red Dent	1	79	Aug. 2	44
Rustler White Dent	1	90	Aug. 4	38
Silver King White Dent	1	102	Aug. 8	80
Wisconsin 7 Silver King	1	89	Aug. 12	75
Wisconsin 25 (Standard)	1	87	Aug. 2	9
Wisconsin 25 (Improved)	4	90	do	36
Wisconsin hybrid (A × Hy) ×R₃	1	112	Aug. 10	85
Flint:				
Gehu North Dakota Yellow Flint	3	52	July 29	2
Kutias	3	107	Aug. 16+	85
Longfellow Yellow Flint	1	82	Aug. 8	40
Manitoba Flint	1	68	July 31	10
Mercer Yellow Flint	1	71	Aug. 2	23
Michigan Flint	1	51	July 29	0
North Dakota White Flint	1	56	do	6
Rainbow Flint	1	86	Aug. 4	21
Smutnose Flint	3	80	Aug. 2	15
Squaw Mixed Colored Flint	1	50	July 29	0
Triumph Yellow Flint	1	80	Aug. 2	30

The correlation between resistance and lateness of the corn (lateness indicated by the silking date), previously established for open-pollinated varieties of sweet corn by other investigators, already mentioned, was confirmed in these studies. The coefficient of simple correlation [4] between resistance and lateness estimated on the basis of the sweet corn varieties employed in these studies was $r = 0.86 \pm 0.018$. Similar correlation was also found among the dent and flint varieties (table 5).

TABLE 3.—*Correlations of both silking date and height with resistance to bacterial wilt of sweet, dent, and flint corn varieties*

Date of silking	Sweet corn			Dent corn [1]			Flint corn		
	Varie-ties	Average maximum height	Average index of resistance	Varie-ties	Average maximum height	Average index of resistance	Varie-ties	Average maximum height	Average index of resistance
	Number	*Inches*		*Number*	*Inches*		*Number*	*Inches*	
July 31, or earlier	32	55	6	2	68	20	5	55	4
Aug. 1 to 4	25	65	25	5	84	34	4	79	22
Aug. 5 to 8	16	76	45	4	91	68	1	82	40
Aug. 9 to 12	9	82	68	3	96	75			
Aug. 13 to 16	8	97	79	3	113	82	1	107	85
Aug. 18, or later	1	120	95						

[1] The 4 dent hybrids listed in table 2 have not been included in this table, only the open-pollinated corns being considered.

A second correlation, between resistance and maximum height of the corn, was found within each varietal group. The tall varieties ordinarily showed greater resistance than the short ones. The coefficient of correlation between resistance and height for the sweet corn varieties, estimated with no regard for the earliness or lateness of the corn varieties, was $r = 0.78 \pm 0.027$. However, since the later varieties are usually taller than the earlier ones, as further discussed in the following paragraph, it was deemed necessary to consider the association of resistance with height only in groups of varieties which had the same silking date. Thus there were 19 sweet corn varieties which had the same silking date, August 2, but which differed in the maximum height they reached, from 38 to 82 inches. An examination of these varieties, listed in table 4, shows that the taller ones as a rule are more resistant than the shorter ones. For instance, Whipple White, Extra Early Adams, and Golden Rod, show considerably greater resistance than Mammoth White Cory, Surprise, Extra Early Golden, and others. Early Sweet variety seems to be a conspicuous exception to the general rule as it showed a low degree of resistance in spite of its relatively great height. The same correlation, between resistance and height, was found within other sweet corn groups as well as within the dent and flint corns (figs. 1 and 2).

[4] In all statistical analyses, including estimates of odds for significance, use has been made of R. A. Fisher's Statistical Methods for Research Workers. Ed. 5, 319 pp., illus. Oliver and Boyd. Edinburgh and London. 1934.

A third correlation, that between lateness and height, already mentioned, has been commonly apparent among all varieties of corn.

FIGURE 1.—Differences in resistance to bacterial wilt between early and late corn varieties: *A*, Early, short, susceptible flint variety, destroyed by bacterial wilt; *B*, late, tall, resistant, dent variety.

Kuleshov (*3*) pointed out this correlation through a study of a world-wide collection of corn varieties of all types. He observed further

that the long-season varieties had a greater number of leaves than the short-season ones, although there were some exceptions to this rule.

FIGURE 2.—Kutlas, a late, tall, flint variety, highly resistant to bacterial wilt: *A*, Control row; *B*, inoculated row.

The coefficient of correlation between height and lateness based on the measurements on the 92 sweet corn varieties employed in the present studies was $r = 0.73 \pm 0.032$.

TABLE 4.—*The relation between height and resistance to bacterial wilt in sweet corn varieties having the same silking date*

Variety	Maximum height	Index of resistance	Variety	Maximum height	Index of resistance
	Inches			*Inches*	
Mammoth White Cory	38	0	Golden Giant, De Lue's	69	66
Surprise	46	0	First of All, Maule's	70	45
Extra Early Golden	47	0	Early Sweet	71	14
Portland Market	52	0	New Jersey Early White	73	44
Early Surprise	52	5	Howling Mob	76	51
Columbia	55	0	Golden Giant	77	46
Carmel Golden	58	8	Golden Rod	80	40
Ford Early	60	15	Extra Early Adams	80	53
Golden Bantam	62	15	Whipple White	82	70
Mayflower	69	37			

The coefficients of the three simple correlations just considered for the sweet corn varieties appear higher than those for inbred strains of the Golden Bantam type as reported by Ivanoff and Riker (*2*). The reason for these differences most probably is the fact that the inbred strains have been tested and selected for resistance during three seasons, and during these processes a great number of the early susceptible and the late resistant strains have been eliminated, while the number of relatively early resistant strains has been enlarged.

A comparison in the resistance of the sweet, dent, and flint corn varieties was made in the light of recent studies. Since it was found that resistance is positively correlated with the lateness and height of most varieties, an attempt was made to determine what influences these two plant characters might have upon the greater resistance of the field corn varieties grown in the Middle West. It should be noted in this connection that lateness and height are not necessarily considered to be the cause of resistance, but only correlated with it. The three varietal groups were arbitrarily divided into six categories on the basis of the progressive lateness of the corn. By this arrangement the resistance of groups of sweet corn varieties of a certain lateness could be compared with the resistance of the dent and flint corn varieties of approximately the same lateness, as shown in table 3. This table reveals that the indexes of resistance of the dent corn varieties for each category are in most cases somewhat higher than those of the sweet and flint corn varieties. The same table also shows that the dent corn varieties of the various categories are from 5 to 19 inches higher than the sweet and flint corn varieties. These differences in height, owing to grouping of a relatively small number of varieties, might easily account for the slightly higher resistance of the dent corns just mentioned. However, when some individual sweet, dent, and flint varieties having exactly or almost exactly the same height and exactly the same silking date, are compared for resistance (tables 1 and 2) the differences in the indexes of resistance among the three varieties become insignificant.

Further statistical analysis of the resistance of the sweet, dent, and flint corn was made by calculating the partial correlation coefficients, in addition to the simple ones, and by testing the various coefficients and the differences between them for significance. The results of these calculations are included in table 5.

TABLE 5.—*Simple and partial correlation coefficients among resistance, silking date, and height of sweet, dent, and flint corn*

Kind of correlation	Plant characters correlated [1]	Sweet corn		Dent corn		Flint corn	
		Number of variables	r (coefficient of correlation)	Number of variables	r (coefficient of correlation)	Number of variables	r (coefficient of correlation)
Simple	RM	92	0.860**	21	0.847**	11	0.980**
	RH	92	.784**	21	.816**	11	.902**
	MH	92	.733**	21	.773**	11	.902**
Partial [2]	RM:H	92	.678**	21	.591**	11	.902**
	RH:M	92	.445**	21	.478*	11	.214
	MH:R	92	.316**	21	.267	11	.214

[1] R = Index of resistance.
M = Maturity (silking date).
H = Height.
*P value (level of significance) exceeds the 5-percent point.
**P value exceeds the 1-percent point.
[2] Correlation between 2 characters when holding the effect of the remaining character constant.

The differences among the simple correlation coefficients of the sweet, dent, and flint corn for the same pair of characters was not found to be significant. Likewise no significant differences were found among the partial correlation coefficients of the three types of corn for the same pair of characters. It appears, therefore, that resistance is correlated with silking date and height to a high, and approximately the same degree, within each type of corn. The relatively higher resistance of the most dent corn varieties grown in the Middle West is not because of any type of resistance inherent in these varieties and lacking in the sweet and flint types, but because of the relatively greater height and lateness characteristic of the dent corn varieties grown in that region.

SUMMARY

Ninety-two sweet, 17 dent, and 11 flint corn varieties were tested for resistance to bacterial wilt by artificial inoculation.

Great differences in resistance were found within each of the three varietal groups of corn.

A high correlation was found between resistance and height, resistance and lateness, and height and lateness in each varietal group.

The dent corn varieties of a certain lateness and height were found to be no more resistant than sweet and flint corn varieties of the same lateness and height.

It appears that the resistance of open-pollinated field corn is similar in type and degree to that of open-pollinated sweet and flint corn.

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RESISTANCE TO BACTERIAL WILT OF INBRED STRAINS AND CROSSES OF SWEET CORN [1]

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INTRODUCTION

The bacterial wilt disease of maize (*Zea mays* L.) caused by *Phytomonas stewarti* (Smith) Bergey et al. in recent years has been one of the most serious diseases of sweet corn in the United States.[3] Attempts to control the disease by developing resistant varieties seem to offer greater promise than other measures, and perhaps the only promise. The present report summarizes the results of 8 years' studies on the resistance to bacterial wilt of more than 2,000 inbred lines and crosses of the Golden Bantam type of sweet corn.

Many reports on bacterial wilt have appeared since Stewart *(17)*[4] discovered the disease in 1895. The first observations on resistance of open-pollinated varieties of corn to bacterial wilt were made by E. F. Smith *(15)* in the summer of 1903, when the disease was very prevalent near Washington, D. C. Smith found that among the approximately 30 sweet-corn varieties tested, infection varied from 0 to 66 percent and that infection was greatest among the early varieties. The late varieties suffered little or no damage. Smith's findings were confirmed by the more extensive trials of Rand and Cash *(12, 13)*. Thomas *(18)*, who tested the resistance of 39 open-pollinated varieties of sweet corn under conditions favorable for natural infection and development of the disease, observed a tendency for the resistant quality to be reduced when selections were made for earlier maturing strains. For instance, while the Fordhook variety showed marked resistance, Early Fordhook under the same conditions showed more than 10 percent infection. Early Adams was somewhat resistant while Extra Early Adams was susceptible. Similar results, with some exceptions noted, were obtained in later studies by Mahoney, Muncie, and Marston *(11)*, Clinton and Singleton *(1)*, G. M. Smith,[5] and others.

Many investigators have worked on the resistance of inbred and hybrid corn to bacterial wilt. Reddy and Holbert *(14)* tested by artificial inoculation the resistance of 15 inbreds of yellow dent corn and 4 of their recombinations, all maturing in approximately the same number of days. The differences in resistance were estimated by the mean percentage reductions in yield of ear corn. The first generation crosses were found to be more resistant than their component inbred strains, and all the progenies of some of the inbred

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lines tested were uniformly high in resistance. On the basis of these results Reddy and Holbert suggested the possibility of developing resistance to bacterial wilt in some of the popular wilt-susceptible varieties of sweet corn. Ivanoff and Riker (*7, 8*) and Ivanoff, Riker, and Dickson (*21, 22*) in preliminary notes reported a correlation between resistance and vigor on the one hand and resistance and lateness on the other, among inbred strains of Golden Bantam sweet corn. They stated that, after taking into consideration the factors for vigor and lateness of the strains, it commonly appeared that crosses between susceptible inbreds gave susceptible hybrids; that crosses between susceptible and resistant inbreds gave resistant hybrids; and that crosses between resistant inbreds gave resistant hybrids. They also reported that hybrid vigor is responsible for some resistance. Wellhausen (*19, 20*) made genetic studies on resistance in 56 inbred lines of field, flint, and sweet corn, and 141 crosses made from these lines. He found that the inbred lines varied in resistance from highly susceptible to highly resistant. The majority of the field-corn inbreds were resistant; the majority of the inbreds of the Evergreen group of strains were intermediate in resistance; and the majority of the early sweet-corn inbreds were susceptible. The crosses tested for resistance were made mostly between highly resistant field-corn inbreds and very susceptible sweet- and flint-corn inbreds. Wellhausen reported that he found resistance to be dominant in all F_1 material tested and that in a few cases the F_1 hybrids were more resistant than either of the parents. He also reported the results of F_2 and backcross tests of two hybrids (highly resistant field-corn parent × very susceptible flint-corn parent, and highly resistant field-corn parent × very susceptible sweet-corn parent) which showed a definite segregation of factors for resistance, with a strong indication that two major dominant complementary genes, with perhaps a third modifying gene, were involved in resistance. However, in making and testing the various crosses for resistance, Wellhausen does not seem to have taken into consideration the usual association of the characters for lateness and height of the host with its resistance, and particularly the lateness and height of the crosses as compared with those of the composing inbreds. Wellhausen also found differences in the reaction of the fibrovascular bundles to bacterial invasion among the different susceptible lines of corn.

Mahoney and Muncie (*10*), in a report of trials on resistance in which they employed inbred strains and hybrids, raise the question whether resistance to bacterial wilt is heritable. Their conclusions seem to indicate that wilt resistance in corn is governed mainly by the vigor and rate of growth of the tested plants, the more vigorous and the more rapidly growing plants being able to outgrow the disease more easily than the less vigorous ones. They also point out the important fact that initial infection is not a measure of resistance, for ordinarily resistant lines in the course of time outgrow the disease.

The purpose of the present investigation is to clarify the principles involved in the resistance of sweet corn to bacterial wilt and thereby to facilitate the development of disease-resistant hybrids. The results herein reported are concerned mainly with the following three phases of the problem: (1) Degree of resistance; (2) correlation of resistance with certain plant characters; and (3) modes of inheritance of resistance. Preliminary accounts of a part of these studies have already been given (*7, 8, 21, 22*).

MATERIALS AND METHODS

Some of the preliminary inoculation tests were started in 1931 on the trial plots of the Wisconsin Agricultural Experiment Station, at Madison, Wis. (*3*). The main resistance trials were started in 1933, and continued through 1934 and 1935. These were conducted near Rochelle and De Kalb, Ill.

The corn used in the experiments was as follows: In 1933, about 430 inbred strains, approximately 200 hybrids, and 30 open-pollinated varieties of sweet corn; in 1934 about 900 inbreds and approximately 350 hybrids and top crosses; and in 1935 about 600 inbreds and 350 hybrids and top crosses and a large number of commercial crosses and open-pollinated varieties. Nearly all of the inbreds and crosses were derived from open-pollinated Golden Bantam corn and about one-third of them were of the true Golden Bantam type, the ears having eight rows of kernels. Most of the Golden Bantam lines used had been inbred for 6 or more years.

Of the corn tested from season to season as a whole the following may be said: The inbreds tested in 1935 were practically the same as were tested in 1933 and 1934, with the exception of those which were eliminated in the course of the trials because of extreme susceptibility and those which were further selected for their resistant qualities. The hybrids, on the other hand, represented to a large degree new material each year. Genetically, so far as resistance is concerned, the hybrids as a group differed considerably from season to season. Thus, the hybrids tested in 1933 and 1934, particularly those tested in 1933, were produced at a time when the resistance of their respective parent inbreds was not well known. These parents consequently in the majority of cases possessed a low degree of resistance, as was shown by the data obtained in 1933 and 1934. On the other hand, the hybrids tested in 1935 were made from inbreds most of which were already known to possess a high degree of resistance. Since the resistance of the hybrids seems to be influenced by the resistance of the parent inbreds, as already reported in preliminary notes by Ivanoff, Riker, and Dickson (*21*, *22*), the hybrids tested in 1935 were on the whole considerably more resistant than the hybrids tested during the previous two seasons. These differences in the hybrid material tested each year must be borne in mind as they have a bearing on the interpretation of some of the results in this report.

In 1933 each line of corn was usually represented by one row of approximately 45 plants, and in 1934 and 1935, by two rows each of about the same number of plants. Some of the lines were replicated from two to five times, others were represented by a larger number of individuals than stated above, while still others, because of unfavorable conditions for germination (particularly during the cold, wet early spring of 1935), had fewer than the usual number.

In addition to the above-mentioned replications, four varieties of corn were replicated from 10 to 30 times throughout the experimental field. These were Golden Gem, Golden Bantam, Purdue Bantam, and Golden Cross Bantam (*16*). The reaction of the replicates to the disease are reported under Preliminary Trials.

The resistance of the corn strains was tested by artificial puncture inoculation and by natural infection, when that occurred. These two methods of testing for resistance seemed to strengthen each

other, and so far no incompatibility between them has been noted, although such incompatibility may sometime occur, as will later be shown. Under the conditions of these trials artificial inoculation was considered to provide a more dependable means for measuring resistance, and during the last two trial seasons it was the only method available.

The plants were inoculated with an instrument described earlier by Ivanoff (4). By the use of this instrument eight men were able to inoculate approximately 100,000 plants in 2 days. The usual method of puncture by means of an ordinary needle was entirely inadequate because progress was too slow and the inoculations thus made were not uniform. The use of a common hypodermic syringe for inoculation of corn plants was found unsatisfactory because the needle plugged frequently, the plunger was slow and inconvenient to operate, and ejection of small uniform amounts of inoculum was difficult. Inoculating a great number of plants in a relatively short time and in a uniform manner is particularly important in resistance trials where small variations in resistance are to be secured. It must be borne in mind that the age of the plant influences the degree of infection considerably and a delay in inoculation of a week or 10 days in many cases may lead to confusing results.

The plants were inoculated by making two punctures at the base of each. The thrusts, which passed entirely through the culm, were made crisscross one above the other about 1 inch apart. In this manner of inoculation the introduction of the bacteria into the plant was well insured.

Some of the plants of each corn strain were inoculated and some were left uninoculated as controls, or for tests through natural infection. In 1933 about two-thirds of each row, or about 30 plants, were inoculated, and about 15 plants were left untreated. In addition, most of the material was replicated on another plot where the strains were subjected to natural infection only. In 1934 and 1935 the plants in one row (about 45) were inoculated, while those in the other row served as checks. In a few cases puncture controls were made with sterilized water instead of bacterial suspension, but the plants so treated showed no difference from unpunctured plants.

In all trials inoculations were made at two different times. The first were made during the most susceptible stage for most of the corn strains, about 5 to 6 weeks after planting, the exact time depending upon the growing conditions and upon the condition of the host. The second inoculations followed the first in about 2 weeks.

Five cultures, representing various types of wilt-producing bacterial organisms, were used to inoculate the plants (3). These cultures were selected to insure infection in susceptible plants with the various diseases known as Stewart's wilt, bacterial wilt, and bacterial stalk rot, as defined by Ivanoff (5). At least three of the five cultures were progenies of single bacterial cells; the others were purified by plating. Three or four of the cultures used were derived from diseased material obtained from the State of Illinois, while one or two of the others, although they occur naturally in Illinois, were isolated from diseased corn plants grown in eastern States. One of the Illinois cultures was usually freshly isolated from naturally diseased material shortly before the inoculations were made. The pathogenicity of the

cultures was tested on corn plants in the greenhouse some days before they were used in the field. The various cultures were grown separately on nutrient dextrose agar plants in 12-ounce bottles, and a mixed standardized suspension made from 3- to 5-day-old cultures was prepared at about ½-hour intervals during the time of inoculation in order to keep the suspension fresh. The turbidity of the suspension was approximately equal to that obtained by mixing 97 cc of 1-percent sulphuric acid solution with 3 cc of 1-percent barium sulphate solution (suspension no. 3 of McFarland nephelometer (9)). Although the degree of pathogenicity of some of these cultures varied on the same host, so far the inoculation tests have not shown that these cultures represent different specialized races such as are known to occur with *Puccinia graminis* Pers. and some other pathogens. Likewise no appreciable antagonism has been noted among the various cultures in the combinations employed.

The following records of the plants were kept: (1) Degree of resistance, (2) length of the period to maturity, and (3) height at three different intervals, including that at the time of maturity.

The degree of resistance of each inoculated plant at a certain time was estimated on the basis of disease injury to the plants. Each plant was given one of the following five indices of resistance: 0, 25, 50, 75, and 100. These corresponded to five more or less distinct degrees of disease injury, estimated (1) by the condition of all the leaves, (2) by the degree of stunting and corresponding ear development, and (3) in some cases, by the condition of the inside stalk tissues. A plant which at the time of record taking was found to be dead, with all of its parts dry and light brown in color on account of the disease, was very stunted, and had a very small ear or no ear at all, received an index of resistance of 0, while a plant which appeared normal, like the uninfected control plants of the same strain (even though the bacteria were present within its tissues), received an index of resistance of 100. Between these two extreme degrees there were plants which exhibited three other marked degrees of wilting. These were given an index of resistance of 25, 50, and 75. Thus a plant which was considerably stunted, with about one-half of its leaves dry and light brown, with its stalk still green, and with a small ear received an index of resistance of 25. Another plant, less stunted, with most leaves green but considerably striped, and with an ear about one-half the size of normal, received an index of 50. A plant only slightly stunted, with all leaves green, mostly free from stripes, with an ear slightly below normal, received an index of 75. The index of resistance for an entire strain was stated as the mean of the indices of all the plants of that strain. These indices corresponded roughly to the percentage yield at the time of harvesting the corn. The final records on resistance were taken at the time that most of the corn ears reached canning stage. This last point is discussed later.

All records were taken by number without reference to the identity of a strain or its past performance.

The length of the period to maturity (the lateness or the earliness) of the inbred lines was measured by the date of silking, and in the case of the hybrids, by both the date of silking and the date of harvesting for canning. The silking date of a line was the date on which 75 percent of the corn plants of that particular line came into silk. This method of determining the earliness or lateness of a corn strain has

been used by Huelsen and Michaels[6] and found to be very satisfactory. Its dependability was confirmed in these studies by a comparison between the silking dates and the harvesting dates for the same strains of corn.

The height of the corn of the different strains was measured at three intervals, that is, at the time of the two inoculations and after the plants had reached their maximum height. The height and maturity measurements were made on the uninoculated control plants.

The degree of reaction to the inoculations of the strains as a whole differed during the 3-year trial. In 1934 and 1935, because of the earlier inoculations, and because of favorable environmental conditions following the inoculations, the plants were affected to a higher degree than in 1933. Correspondingly, the average index of resistance of the strains in 1934 and 1935 was considerably lower than in 1933 for the same strains. However, on the whole the relative resistance of the various strains during the three seasons' trials was maintained. Thus as a rule strains which showed a relatively high index of resistance in one season showed a relatively high index of resistance in the other two seasons. Likewise, strains which were very susceptible in 1933 were among the first to go down with the disease in 1934 and 1935.

Unless otherwise stated the indices of resistance of the various inbreds and hybrids given in this paper are those for 1934.

The term "resistance" as used in this paper implies the ability of the host plant to continue a more or less normal development in the presence of the pathogenic bacteria within its tissue. Medium-resistant and high-resistant lines mentioned in this paper indicate relative degrees of host resistance as defined above. The term "resistance" excludes cases of immunity. No immune corn plants have yet been found; i. e., no plants after being either inoculated at an early stage or subjected to conditions favorable for natural infection at an early stage, have failed to develop leaf stripes on the first few leaves.[7]

PRELIMINARY TRIALS

The results of a number of earlier inoculation trials, together with some observations made in the course of these studies, because of their preliminary character, are reported at the outset. They contribute toward a better understanding of the methods employed and of the interpretation of data given in Experimental Results. These trials and observations were on: (1) Comparative efficiency of various methods of inoculation, (2) period of greatest host susceptibility, (3) age of host plant in relation to type of symptom expression, (4) comparative rate of wilting of hybrids and their respective inbred parents, (5) determination of time for final record taking, (6) uniformity of inoculation, and (7) comparison between artificial puncture inoculation and natural infection. The detailed experimental data are omitted because of their volume.

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[6] HUELSEN, W. A., and MICHAELIS, W. H. THE EFFECT OF MATURITY ON THE YIELD COMPLEX OF SWEET CORN. (Unpublished manuscript.)

[7] High degrees of resistance to the disease have been shown by nearly all the sorghum, common millet, German millet, Sudan grass, and yellow foxtail plants tested thus far (5). The plants usually developed leaf stripes and occasionally some symptoms in the culm when artificially inoculated at an early stage, but they soon recovered with little or no apparent effect on yield or vigor. On the other hand, some varieties of wheat, barley, oats, sugarcane, and other grasses appeared immune to the disease, as they developed no leaf or other symptoms following inoculation.

A number of different methods of inoculation were tried on open-pollinated Golden Bantam corn in the greenhouse and the field. These methods consisted of (1) applying drops of inoculum on the tips of the leaves and in the spiral whorl of young seedlings, (2) atomizing punctured and unpunctured leaves, (3) smearing the inoculum on the seeds, (4) puncture-inoculating the kernels, and (5) puncture-inoculating the young plants at the base. The various trials were repeated three or more times and involved from 300 to several thousand plants. Of all the methods tested, puncture-inoculating the young plants at the base was the most effective. By this method many thousands of young plants were inoculated at a susceptible stage and in no case did the characteristic leaf stripe symptoms (*3*) fail to appear. These stripe symptoms, however, may fail to become evident, or may easily be overlooked in cases when (1) the plants have become infected at a late stage, (2) the plants have become infected at a very early stage (about a week or so after planting) and rot-producing bacteria, other than, or in addition to *Phytomonas stewarti*, have established themselves in the plant tissue, (3) the wilt bacteria after entering a seedling plant have failed to establish themselves systemically before the emergence of the tassel (delayed systemic infection), and (4) in exceptionally susceptible strains, the discoloration of the entire leaf tissue has taken place at an extremely rapid rate. These cases, some of which are relatively rare in nature, have been avoided in the resistance studies by adapting the methods of inoculation already described.

The period during which the corn plant is most susceptible to infection induced by puncture inoculation at the base of the plant was determined. Through a series of trials during five seasons, in which several corn varieties and inbred strains were tested, it was found that the plants were most susceptible when 3 to 6 weeks old. Later inoculations with some varieties resulted in a distinctly lower degree of infection. On the other hand, if the inoculations were made when the plants were very young, 1 to 2 weeks old, there was occasionally a low degree of infection, delayed systemic infection, or none. This probably was because of the small number of leaves punctured, which, at that stage of host development, withered and died before enough, if any, of the bacteria were able to reach the stem bud tissue. It was not so much the age of the plant as its degree of development that was important for susceptibility. Cold weather may retard the development of the growing plant considerably, in which case plants 5 weeks old may have reached only their five-leaf stage. In such cases later inoculations gave better results. Inbreds and hybrids of the Golden Bantam type were found to be very susceptible in their seventh- to tenth-leaf stage, when 16 to 20 inches high. At this stage the stem tissue is usually just emerging above the soil line. In the resistance trials a great many strains of corn were inoculated some of which developed more slowly than others. The corn plants were therefore inoculated twice during the growing period so as to make sure of striking the period of greatest susceptibility of each line at least once.

The age of the plants was found to determine not only the degree of infection, but also the type of symptoms that developed. Corn plants inoculated before the emergence of the tassel developed the characteristic leaf-stripe symptoms of the disease. These symptoms appeared

whether the puncture passed through the leaf sheaths and the unfold-ing leaves alone, or through the tissue of the growing stem as well. In older plants, after the tassel and all the leaves had emerged, the leaf stripes usually did not appear following inoculation at the stem. The leaves of the susceptible corn strains inoculated at this late stage with-ered gradually and the color changed from green to light brown, whereas the leaves of the highly resistant strains remained more or less normal in color. Naturally infected plants of relatively susceptible open-pollinated varieties in some seasons exhibited a gradual, diffuse type of wilting without showing the characteristic leaf stripes. Such plants are commonly considered either to have become infected at a late stage, or, more rarely, to represent cases of delayed systemic infection from very early infection.

The rate of wilting, independent of the degree of resistance, of the hybrids in relation to that of their respective inbred parents was investigated because of its importance in determining the time for taking the final records on resistance and in interpreting the results. Consequently, tests were undertaken with about 100 hybrids to determine the respective rate of wilting of hybrids and parents under conditions of severe infection, the latter being measured by the per-formance of standard corn lines.[8] The results showed, that in most cases there was no appreciable difference in the rate of wilting of parents and hybrids throughout the season. Such differences may be brought about, however, in the case of susceptible lines and hybrids if the inoculations have not been severe enough.

The time for taking the final records is important in the evaluation of the strains for resistance. It was thought desirable to delay the record taking as much as possible in order to allow the bacteria to have full play in association with the host. Since the first symptoms following inoculation appeared on the leaves of both susceptible and resistant strains, too early record taking tended to equalize the differences among the strains. Later in the season these differences usually became more apparent, the susceptible strains gradually going down with the disease and the resistant strains gradually coming out of it. On the other hand, too great a delay in taking the notes on resistance may lead to difficulties and even confusion because of the natural discoloration and withering of the leaves at later stages of plant maturity. It was found that the best time for taking the final records on resistance was when the corn reached canning stage, which for the Golden Bantam variety, was about 70 to 75 days after planting, and about 40 to 45 days after the first inoculation. By that time the disease on the various corn strains had reached a more or less station-ary stage of development.

The uniformity of inoculation was demonstrated by the more or less uniform degree of infection produced in highly inbred lines, in related lines, and in replications of certain lines of corn. In the very sus-ceptible lines practically all of the individual plants died at the same rate throughout the season, and in the highly resistant lines, the slight wilting was approximately the same in each plant. Among the plants of certain intermediate lines some variations in resistance were observed. In the summer of 1934, however, there were exceptions to

[8] By severe infection is meant infection which causes more than a 75-percent loss in yield of open-pollinated Golden Bantam corn at time of harvesting for canning. Other varieties and crosses, such as Golden Gem, Purdue Bantam, and Golden Cross Bantam have also been used to measure severity of infection.

these rules, evidently because of the presence of other diseases toward the end of the season, such as smut and fungus stalk rots. There were some variations in the reaction to the inoculations among the several replications of certain lines. The index of resistance of about 15 replications of a line varied from 0 to 10 points from the mean, most of the values being distributed closely around the mean. The more conspicuous variations, however, were probably due more to the effect which soil and land incline may have upon the host than to the inoculation technique, for when two or more rows of the same line of corn were planted near each other, the variations among them were hardly noticeable.

The correlation between infection produced by artificial inoculation of certain lines of corn and that occurring naturally in the field in the same lines of corn was of particular importance in these studies. Since bacterial wilt of corn is epidemic in character, occurring in some seasons to a considerable degree and being almost entirely absent in other seasons in the same locality, artificial inoculation in the continued testing for resistance of corn lines was necessary. Such inoculation would be of doubtful value in measuring resistance, however, if the infection thus induced in relatively resistant and susceptible lines of corn did not correlate with the infection produced in the same lines of corn through natural agencies. Such correlation was demonstrated to exist in 1933, when natural infection was widespread early in the season in fields of Golden Bantam corn as well as in the experimental plot. Thus inbred strains in which the inoculated plants had an index of resistance of 0 or near 0 showed the highest degree of injury from natural infection. Likewise, many strains which had a high index of resistance remained relatively unaffected by the disease through natural infection. Among the commercial varieties of corn the same relation existed. Thus, Country Gentleman, Golden Cross Bantam, Golden Bantam, and Golden Gem showed in this order decreasing degrees of resistance both from artificial inoculation and from natural infection. The same varieties have been known by corn growers to hold the same relative position in regard to resistance during previous years when the disease has been widely prevalent. Additional evidence on the correlation of results obtained by artificial inoculation and natural infection was gathered in the summer of 1935, when approximately 25 open-pollinated varieties, previously tested for resistance through natural infection in three different seasons by Rand and Cash (*12, 13*), were again tested by artificial inoculation (*6*). When these varieties were arranged in the order of their resistance, as determined by artificial puncture inoculation, the order was found to approximate that for the same varieties made by Rand and Cash on the basis of natural infection.

As a method of testing resistance natural infection has some serious disadvantages, such as (1) it takes into account only the strains of the causal bacteria that happen to be present locally; (2) it may occur in widely varying degrees from season to season, and at different places in the same field during the same season (*13*); (3) the conditions for its severest development may occasionally occur after the period of greatest susceptibility for some strains, in which case early susceptible strains may escape damage, while later, and relatively more resistant, strains may show considerable injury (*10*); and (4) individual plants even at their most susceptible stage may escape the disease. Arti-

ficial inoculation was found to have the following advantages over natural infection: (1) Infection was induced at the most susceptible stage of the host; (2) all plants became infected at the same time; (3) infection was uniform; (4) escapes were avoided; (5) the type and amount of the inoculum was standardized; and (6) the inoculating suspension contained representatives of all the known types of the causal bacteria. By artificial inoculation severe infection was induced even when the conditions for natural incidence of disease were very unfavorable, as was the case in 1934 and 1935.

EXPERIMENTAL RESULTS

The experimental results reported here are of representative trials and give the general summary of three seasons' studies, mainly on the resistance of the inbred lines and experimental hybrids. For the sake of brevity, detailed accounts of many repeated experiments have been omitted. For the same reason it has been thought advisable to give as representative the tabulated data from 1 year's trials (1934), supplementing these data by those of the other two seasons' trials only when necessary for further clarity.

FIGURE 1.—A number of inbred strains of sweet corn inoculated with wilt-producing bacteria. Some of these strains tolerated the activities of the bacteria better than others.

Because of the similarity in the results between the F_1 hybrids and top crosses in regard to the general principles governing resistance, no tabulated data on the top crosses have been included.

RESISTANCE OF INBRED LINES

Significant differences in resistance among the inbred lines of sweet corn were found (fig. 1). Some of the strains wilted and died shortly after inoculation without being able to produce ears, while others reached maturity without any appreciable loss in either yield or vigor. The majority of the inbred strains, however, showed degrees of resistance intermediate between these extreme limits.

The taller strains as a rule showed greater resistance than the shorter strains, as may be seen from table 1.[9] In this table each of three groups of inbreds, comprising relatively early- medium- and late-maturing strains (as determined by the silking date), has been divided into four classes according to the maximum height they reached and the average index of resistance. It is evident that as the height of the inbreds increases from class to class, so does the index of resistance for each class. Table 1 shows that there is a definite correlation between the height of the strains and their resistance, and, correspondingly, that a resistant line is more likely to be found among the tall-growing, vigorous inbreds than among the short, weak ones (fig. 2). However, there were some tall-growing and vigorous-appearing strains (also included in the table) which showed little resistance to the disease (fig. 3). It appears, then, that one cannot be sure of the resistance of any particular tall and vigorous inbred, unless it has been thoroughly tested.

TABLE 1.—*Relationship between height and date of silking, respectively, with index of resistance of sweet corn inbreds of the Golden Bantam type, in 1934* [1]

Silking date [3]	Index of resistance for inbreds of maximum height indicated [2]								Total or average	
	35 inches or below		36 to 47 inches		48 to 59 inches		60 inches or above			
	In-breds	Index of resist-ance	In-breds	Index of resist-ance	In-breds	Index of resist-ance	In-breds	Index of resist-ance	In-breds	Index of resist-ance
	Num-ber		*Num-ber*		*Num-ber*		*Num-ber*		*Num-ber*	
Aug. 9 or earlier	4	10	50	29	49	36	4	71	107	31
Aug. 13	6	31	107	34	71	50	18	61	202	42
Aug. 21 or later	3	22	32	37	26	55	7	72	68	47
Total or average	13	22	189	33	146	46	29	65		

[1] Similar results were secured in 1933 and 1935.
[2] The average height of the 4 groups of inbreds at the time of the first inoculation (5 weeks after planting) was 15, 16, 17, and 18 inches, respectively. These slight differences in height are not considered to have significant influence on the final results.
[3] All corn planted June 11.

The later inbreds as a rule showed greater resistance than the earlier ones. This relation between lateness and resistance has been observed by other workers, already mentioned, in a number of open pollinated varieties of corn. These workers, however, have not taken into consideration the height of the plants, and a correlation between lateness and resistance cannot be very exact unless it is made among strains of approximately the same height and type, as will be explained later. In table 1, such a correlation is shown within the groups of inbreds of approximately the same height that are large enough to have statistical significance.

There were several relatively early strains among all those tested, the resistance of which was not correlated with height or lateness or, so far as could be determined, with any other plant character. These strains were approximately as early as open-pollinated Golden Ban-

[9] Height at maturity has been generally considered to be the best single measure of the vigor of a strain of corn, but since not every tall strain could be considered as vigorous, the writers prefer to use height rather than vigor when correlating this plant character with resistance.

tam, were not very tall, but showed a high degree of resistance. The resistance of such strains, for lack of a better term, has been called "true resistance." This resistance may be considered as a distinct type and may exist in different degrees within most of the strains. The degree of this type of resistance, within a particular late corn

FIGURE 2.—Two inbred lines of sweet corn inoculated with wilt-producing bacteria. The inbred line on the right was tall and showed a considerably higher degree of resistance than the short line on the left. A high correlation was found between resistance and height of the inbred lines.

strain, however, would be difficult to determine on account of the masking effect of the characters of height and lateness and the resistance associated with them.

In addition to the correlation between resistance and height and between resistance and lateness, a third correlation was found among

certain groups of inbred strains between height and lateness. This correlation is an important factor in resistance trials because of its heretofore confusing influence in the matter of determining the exist-

FIGURE 3.—Two tall and vigorous inbred lines of sweet corn of approximately the same height and maturity inoculated with wilt-producing bacteria. The one on the left was very susceptible, while the one on the right was very resistant. Tall, vigorous inbreds susceptible to the disease are rare.

ence and extent of the two distinct correlations in resistance just mentioned. However, its interference in the interpretation of the results of these studies has been avoided by segregating the strains

in groups according to lateness and height, as shown in table 1, and by the use of partial correlations.

Simple, partial, and multiple correlation coefficients were calculated for the characters of resistance, lateness, and height of approximately 860 inbred strains tested in 1934.[10] The simple correlations between resistance and height on the one hand, and resistance and lateness on the other were 0.496 and 0.519, respectively. The partial correlation coefficients for the same pairs of variables when holding the effect of the remaining variable constant, were respectively 0.643 and 0.674. In all four cases the value of P (level of significance) exceeds the 1 percent point. The multiple correlation coefficient for these inbred lines was $R = 0.768$, indicating that the resistance of the average inbred is highly correlated with its height-lateness character complex, and that there is an additional degree of resistance that is not correlated either with height or lateness.

RESISTANCE OF F₁ HYBRIDS

The hybrids, like the inbreds, varied greatly in resistance to bacterial wilt. Some were as susceptible as the open-pollinated Golden Bantam variety and succumbed to the disease early in the season, while others reached the canning stage without any appreciable loss in yield or vigor.

An attempt was made to determine the principles governing hybrid resistance by analyzing the data gathered in the three seasons' trials. Attention was directed particularly to the possible correlation of resistance with height and lateness, to inheritance of resistance, and to the dominance relationships of resistance. The plan of presenting the data on hybrid resistance is briefly as follows: (1) Influence of height and lateness of hybrids on hybrid resistance; (2) influence of inbred parents' resistance upon hybrid resistance; (3) the dominance relationships of resistance; and (4) certain important exceptions to the general rules regarding hybrid resistance.

Because of the great number of hybrids studied (approximately 1,000), it is not feasible to consider each individually. Instead, detailed consideration is given only to a relatively small group of representative hybrids by means of which the major points are illustrated. This study is supplemented by a group study of the hybrids as a whole. The 36 hybrids comprising this representative group each had one inbred parent, *M*, in common. Thus they were genetically more closely related than the hybrids as a whole; they showed considerable differences in height, lateness, and index of resistance.

INFLUENCE OF HEIGHT AND LATENESS OF HYBRIDS ON HYBRID RESISTANCE

The relation of hybrid resistance to height and lateness is considered in the representative group of hybrids listed in table 2. These hybrids are divided first into tall and short, and later into early and late, the dividing line being the average value for height and the date of harvesting for canning, respectively. It appears from the indices of resistance that on an average the taller hybrids are more resistant than the shorter, and the late more resistant than the early. Thus the tall hybrids have an average index of resistance of 43 and the short

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[10] In all statistical analyses use has been made of Fisher's Statistical Methods (*2*).

hybrids one of 37.   Likewise the early hybrids have an index of resistance of 25 and the late hybrids one of 50.   The differences in the indices of resistance between the groups would undoubtedly have been greater if only the extreme cases had been considered.   While it appears that height and lateness are generally associated with greater resistance, as was noted among the inbreds, there are still significant differences in the indices of resistance, up to 56 units, among the individual hybrids of each group.   However, when the same 36 hybrids were again classified into two groups, this time on the basis of the two combined plant characters, namely, into tall, late hybrids and short, early hybrids, the differences in the indices of resistance among the individual hybrids within each group became somewhat narrower.   (The table showing this grouping, for the sake of brevity has been omitted.)   Yet significant differences in resistance among the individual hybrids within each group still existed which could not be accounted for by the combined effect of the height and lateness characters alone.   It appears, therefore, that still another factor or factors associated with resistance must be sought.

INFLUENCE OF INBRED PARENTS' RESISTANCE UPON HYBRID RESISTANCE

Next the possible influence of the inbred parents upon the hybrids is considered.   In table 3 the same 36 hybrids are reclassified into three groups on the basis of the degree of resistance of the parent inbred which, together with parent M, went into the making of each of these hybrids.   The index of resistance of the common parent M was 36, while that of the other parents varied in the three groups respectively from 0 to 25, 26 to 50, and more than 50.   The average index of resistance for the hybrids of each group was 35, 34, and 61 respectively.   The data indicate that, on the average, the resistance of the hybrids is dependent on the resistance of the inbred parents in general, and upon the more resistant of the two parents in particular.   (Additional data concerning this point are presented later.)   However, as in table 2 the individual hybrids in each group differ considerably among themselves in regard to resistance.   But it has already been brought out that the resistance of this particular group of hybrids is correlated in addition with the height and lateness of the hybrids.   It seems desirable, therefore, to examine the data with these three characters separated.

The evidence presented so far suggests that as a rule the resistance of a hybrid is influenced by at least three major factors, viz, height and lateness of the hybrid and resistance of the parents from which the hybrid was made.   Continuing this line of analysis, each group of hybrids in table 3, in which the hybrids have already been classified on the basis of parental resistance, was further arranged on the basis of height and lateness into four subgroups as follows: Tall-late, short-late, tall-early, and short-early.   The results of this arrangement are shown in table 4.   From table 4 it is seen that, as a rule, the most resistant hybrids fall in the tall-late subgroup, the least resistant in the short-early subgroup, and the rest in the two intermediate groups.

TABLE 2.—*Influence of height and lateness on the resistance to bacterial wilt in sweet-corn hybrids produced by crossing inbred strain M with various other inbred strains of the Golden Bantam type, and tested in 1934*[1][2]

TALL HYBRIDS

Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
		Inches					*Inches*		
19	25	63	Aug. 23	37	137	25	58	Aug. 25	25
49	0	56	Aug. 24	25	182	65	60	Sept. 1	56
71	19	57	Aug. 29	56	187	36	56	Aug. 23	25
74	25	56	...do....	41	189	36	55	Aug. 24	19
111	37	57	...do....	57	412	10	62	Aug. 21	25
134	72	66	Sept. 1	75					
135	56	62	...do....	75	Average	34	59	Aug. 27	43

SHORT HYBRIDS

Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
66	25	53	Sept. 1	65	190	45	47	Aug. 22	25
86	36	51	Aug. 27	35	191	42	53	Aug. 24	30
99	56	52	Aug. 29	56	192	34	52	...do....	30
145	52	45	Aug. 25	63	194	34	50	Aug. 25	30
150	32	54	...do....	49	195	0	51	Sept. 1	30
174	25	51	Aug. 29	35	196	29	51	Aug. 29	42
176	62	54	Aug. 25	50	197	41	50	Aug. 22	10
179	43	51	Aug. 27	62	199	41	52	Aug. 24	25
180	75	46	Aug. 29	59	201	15	48	Sept. 4	45
181	72	53	Aug. 24	56	202	42	51	Aug. 24	35
184	25	54	...do....	10	365	31	51	Aug. 22	25
185	29	47	Aug. 22	10					
186	10	45	Aug. 24	10	Average	37	50	Aug. 26	37

EARLY HYBRIDS

Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
19	25	63	Aug. 23	37	191	42	53	Aug. 24	30
49	0	56	Aug. 24	25	192	34	52	...do....	30
181	72	53	...do....	56	197	41	50	Aug. 22	10
184	25	54	...do....	10	199	41	52	Aug. 24	25
185	29	45	Aug. 22	10	202	42	51	...do....	35
186	10	45	Aug. 24	10	365	31	51	Aug. 22	25
187	36	56	Aug. 23	25	412	10	62	Aug. 21	25
189	36	55	Aug. 24	19					
190	45	45	Aug. 22	25	Average	32	53	Aug. 23	25

LATE HYBRIDS

Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
66	25	53	Sept. 1	65	174	25	51	Aug. 29	35
71	19	57	Aug. 29	56	176	62	54	Aug. 25	50
74	25	56	...do....	41	179	43	51	Aug. 27	62
86	36	51	Aug. 27	35	180	75	46	Aug. 29	59
99	56	52	Aug. 29	56	182	65	60	Sept. 1	56
111	37	57	...do....	57	194	34	50	Aug. 25	30
134	72	66	Sept. 1	75	195	0	51	Sept. 1	30
135	56	62	...do....	75	196	29	51	Aug. 29	42
137	25	58	Aug. 25	25	201	15	48	Sept. 4	45
145	52	45	...do....	63					
150	32	54	...do....	49	Average	39	54	Aug. 29	50

[1] The average value for height of these hybrids was used to divide them into tall and short. Likewise the average harvesting date for canning was used to divide them into early and late. The differences in resistance among the various classes would have been considerably greater if only the extreme cases for height and lateness had been considered.

[2] The average index of resistance of inbred strain M was 36, and its average maximum height was 51 inches.

TABLE 3.—*Influence of the resistance in parent inbreds on the resistance of hybrids when strain M was crossed with various other inbreds of the Golden Bantam type* [1]

GROUP 1, HYBRIDS PRODUCED BY CROSSING INBRED M WITH OTHER INBREDS WITH AN INDEX OF RESISTANCE OF 0 TO 25

Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
		Inches					*Inches*		
19	25	63	Aug. 23	37	184	25	54	Aug. 24	10
49	0	56	Aug. 24	25	186	10	45	...do	10
66	25	53	Sept. 1	65	195	0	51	Sept. 1	30
71	19	57	Aug. 29	56	201	15	48	Sept. 4	45
74	25	56	..do..	41	112	10	62	Aug. 21	25
137	25	58	Aug. 25	25					
174	25	51	Aug. 29	35	Average	17	55	Aug. 28	34

GROUP 2, HYBRIDS PRODUCED BY CROSSING INBRED M WITH OTHER INBREDS WITH AN INDEX OF RESISTANCE OF 26 TO 50

86	36	51	Aug. 27	35	192	34	52	Aug. 24	30
111	37	57	Aug. 29	57	194	34	50	Aug. 25	30
150	32	54	Aug. 25	49	196	29	51	Aug. 29	42
179	43	51	Aug. 27	62	197	41	50	Aug. 22	10
185	29	47	Aug. 22	10	199	41	52	Aug. 24	25
187	36	56	Aug. 23	25	202	42	51	...do	35
189	36	55	Aug. 24	19	365	31	51	Aug. 22	25
190	45	47	Aug. 22	25					
191	42	53	Aug. 24	30	Average	37	52	Aug. 25	32

GROUP 3, HYBRIDS PRODUCED BY CROSSING INBRED M WITH OTHER INBREDS WITH AN INDEX OF RESISTANCE OF ABOVE 50

99	55	52	Aug. 29	56	180	75	46	Aug. 29	59
134	72	66	Sept. 1	75	181	72	53	Aug. 24	56
135	56	62	...do	75	182	65	60	Sept. 1	56
145	52	45	Aug. 25	63					
176	62	54	...do	50	Average	64	55	Aug. 29	61

[1] See footnote 2, table 2.

TABLE 4.—*Influence of height and lateness on resistance to bacterial wilt in hybrids from parent M crossed with inbreds having low (0 to 25), intermediate (26 to 50), and high (more than 50), indices of resistance* [1]

LOW—0 TO 25

Kind of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
			Inches		
Tall-late	71	19	57	Aug. 29	56
	74	25	56	...do	41
Average		22	57	...do	48
Short-late	66	25	53	Sept. 1	65
	174	25	51	Aug. 29	35
	195	0	51	Sept. 1	30
	201	15	48	Sept. 4	45
Average		16	51	Sept. 1	43
Tall-early	19	25	51	Aug. 23	37
	49	0	56	Aug. 24	25
	137	25	58	Aug. 25	25
	412	10	62	Aug. 21	25
Average		15	57	Aug. 23	28
Short-early	184	25	54	Aug. 24	10
	186	10	45	...do	10
Average		18	50	...do	10

[1] See footnotes 1 and 2, table 2.

TABLE 4.—*Influence of height and loteners on resistance to bacterial wilt in hybrids from parent M crossed with inbreds having low (0 to 25), intermediate (26 to 50), and high (more than 50), indices of risistance*—Continued

INTERMEDIATE—26 TO 50

Kind of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
			Inches		
Tall-late	111	37	57	Aug. 29	57
Short-late	86	36	51	Aug. 27	35
	179	43	51	...do.....	62
	196	29	51	Aug. 29	42
Average		36	51	Aug. 28	46
Tall-early	150	32	54	Aug. 25	49
	187	36	56	Aug. 23	25
	189	36	55	Aug. 24	19
	191	42	53	...do....	30
Average		37	55	...do.....	30
Short-early	185	29	45	Aug. 22	10
	190	45	45	...do.....	25
	192	34	52	Aug. 24	30
	194	34	50	Aug. 25	30
	197	41	50	Aug. 22	10
	199	41	52	Aug. 24	25
	202	42	51	...do.....	35
	365	31	51	Aug. 22	25
Average		37	49	Aug. 23	23

HIGH—MORE THAN 50

Kind of hybrid	Plot no. of hybrid	Index of resistance of other inbred	Maximum height of hybrid	Date of harvesting for canning	Index of resistance of hybrid
Tall-late	134	72	66	Sept. 1	75
	135	56	62	...do.....	75
	182	65	60	...do.....	56
Average		64	63	...do.....	68
Short-late	(¹)				
Tall-early	(²)				
Short-early	99	56	52	Aug. 29	56
	145	52	45	Aug. 25	63
	176	62	54	...do....	50
	180	75	46	Aug. 29	59
	181	72	53	Aug. 24	56
Average		63	50	Aug. 26	56

¹ None.

A similar type of analysis was applied to all of the F_1 hybrids tested in 1934 for which complete data were available and which could thus be classified. The results are summarized in table 5, where the hybrids are first divided into four classes on the basis of height and lateness, as tall late, tall early, short late, and short early. Further, each of the four classes of hybrids is divided into three groups, on the basis of the degree of resistance of the inbred parents composing the hybrids. The average measurements of height, harvesting date, and index of resistance for each group of hybrids is given for the purpose of making exact comparisons in determining the effect of a single factor on resistance. An examination of this table shows as a rule (1) that the taller and the later the hybrids are, the more resistant they are, and (2) that when the factors of height and lateness are about equal, hybrids produced from high-resistant inbreds are more resistant than hybrids produced from low-resistant inbreds.

TABLE 5.—*Influence of height and lateness of the hybrids upon hybrid resistance, and of parents' resistance upon hybrid resistance, in 229 hybrids tested for resistance to bacterial wilt in 1934* [1]

Kind of hybrid	Hybrids	Maximum height of hybrid	Date of harvesting for canning	Average index of resistance
	Number	*Inches*		
Tall late: [1]				
A, inbreds with index of resistance of 0 to 25	5	58	Aug. 29	28
B, index of resistance of one inbred 0 to 50 and the other 26 to 50	15	58	---do-----	49
C, inbreds one or both of which had index of resistance higher than 50	19	61	Aug. 30	62
Total or average	39	60	Aug. 29	53
Short late:				
A, inbreds with index of resistance of 0 to 25	3	49	Aug. 30	13
B, index of resistance of one inbred 0 to 50 and the other 26 to 50	30	51	---do-----	40
C, inbreds one or both of which had index of resistance higher than 50	17	49	Aug. 29	55
Total or average	50	50	Aug. 30	43
Tall early:				
A, inbreds with index of resistance of 0 to 25	31	60	Aug. 22	28
B, index of resistance of one inbred 0 to 50 and the other 26 to 50	26	60	Aug. 23	31
C, inbreds one or both of which had index of resistance higher than 50	12	60	Aug. 25	42
Total or average	69	60	Aug. 23	31
Short early:				
A, inbreds with index of resistance of 0 to 25	16	51	Aug. 22	17
B, index of resistance of one inbred 0 to 50 and the other 26 to 50	44	49	Aug. 23	30
C, inbreds one or both of which had index of resistance higher than 50	11	52	Aug. 25	44
Total or average	71	50	Aug. 23	29

[1] See footnote 1, table 2.

The data obtained for the group of 36 hybrids previously mentioned were further analyzed by the use of correlation coefficients. Simple and partial correlations for the various plant characters are presented in table 6. It appears that for this group of hybrids there is a positive correlation between resistance of the hybrids and (1) maturity of the hybrids, (2) resistance of the more resistant of the two parents, and (3) height of the hybrids, the significance of these correlations being in the order named. It is very likely that the value and significance of the correlation coefficients may change with the different hybrid groups under investigation. The multiple correlation coefficient for this group of hybrids is $R=0.866$ (or $R^2=0.735$), which indicates that approximately 73 percent of the resistance of the average hybrid of this group is correlated with the three hybrid characters mentioned above.

TABLE 6.—*Simple and partial correlation coefficients among the various characters of a group of 36 F₁ hybrids* [1]

Simple correlations		Partial correlations	
Variables correlated [2]	r (coefficient of corre- lation)	Variables correlated	r (coefficient of corre- lation)
RhRp	[3] 0.600	RhRp:HhMh	[3] 0.634
RhHh	.297	RhHh:RpMh	.314
RhMh	[3] .720	RhMh:RpHh	[3] .737
RpMh	.254	RpMh:RhHh	−.169
RpHh	.084	RpHh:RhMh	[4] −.339
HhMh	.140	HhMh:RhRp	−.160

[1] A group of hybrids having a common parent, listed in tables 2 and 3.
[2] Rp=index of resistance of the more resistant of the 2 parents; Rh=index of resistance of the hybrids; Hh=height of hybrids; Mh=maturity (harvesting date) of hybrids.
[3] P value (level of significance) exceeds 1 percent point.
[4] P value (level of significance) exceeds 5-percent point.

DOMINANCE RELATIONSHIPS OF RESISTANCE

INSTANCES OF RESISTANCE DOMINANCE

Evidence that the resistance of the parent inbreds is transmitted to the F₁ hybrids in most cases as a dominant is further considered through studies both on groups of hybrids and on individual hybrids. One instance has already been mentioned in connection with table 3 where the average index of resistance of each of the three groups of hybrids appeared to follow closely that of the more resistant parent. More examples of dominance of resistance in groups of hybrids are presented later. In examining the various hybrids for the dominance relationships, one should consider at the outset (1) the height and lateness of each parent and the varying effect of these parent characters upon the height and lateness of the hybrids, and (2) the effect of height and lateness of the hybrids upon the resistance of the hybrids. The interplay of these commonly interrelated characters within a certain individual hybrid, together with the different extent of heterosis exhibited by different hybrids, may often obliterate the expression of resistance dominance, while in another individual hybrid they may create a false appearance of dominance. It seems that because of the interaction of the various resistance factors and because of some exceptions, evidence obtained as a result of analysis based on relatively large hybrid groups as a whole may be more valuable in demonstrating the general principles of resistance than evidence based on the examination of only one or a few hybrids. However, this second approach is not to be ignored, and a study of dominance of resistance has been made in some inbreds and hybrids selected for the purpose.

Thus, individual hybrids in which resistance appeared dominant are shown in table 7. These are the only hybrids among all those tested in 1934 which could be analyzed for resistance dominance without the confusing influence of the characters of height and lateness of both parents and hybrids. Thus, in the case of hybrid 182, both parents are of the same class, namely, tall-late, and produced a hybrid also of the

same class. Both parents had the same silking date, were approximately of the same height, and produced a hybrid with the same silking date and of approximately the same height. The only difference between the two parents was in the degree of resistance, one having an index of resistance of 36 and the other of 65. The hybrid had an index of resistance of 56, a figure closer to that of the resistant parent than to that of the susceptible, thus indicating the dominant character of resistance. The next two hybrids (95 and 265) in table 7 show resistance dominance even more clearly. Hybrids 22, 24, and 25 are short-early, all very susceptible (the last two are reciprocal), and were made from short-early susceptible inbreds. Among the group of hybrids produced from short-early, low-resistant inbreds, there were some which showed an appreciable degree of resistance. These same hybrids, however, were relatively tall and could not be classed as short-early. The resistance of these hybrids was correlated with the increased height resulting from the hybridization. ·

TABLE 7.—*Dominance of resistance to bacterial wilt shown in selected individual hybrids and their parents in which the confusing influence of height and lateness have been eliminated*

Plot of hybrid	Type of inbreds from which the hybrids were made	Index of resistance of—	
		Inbreds	Hybrids
182	Tall-late, high index of resistance	65	56
	Tall-late, low index of resistance	36	
95	Short-late, high index of resistance	56	55
	Short-late, low index of resistance	25	
265	Short-early, high index of resistance	62	61
	Short-early, low index of resistance	30	
22	Short-early, low index of resistance	0	0
	do	0	
24	Short-early, low index of resistance	0	0
	do	0	
25	Short-early, low index of resistance	0	0
	do	0	

CUMULATIVE EFFECT OF PARENTS' RESISTANCE IN RELATION TO DOMINANCE

The question whether the resistance of the hybrids is influenced only by the more resistant of the two parents or by the combined resistance of the two parents was given further consideration. The data on a randomized group of 44 unrelated hybrids tested in 1935 is shown in table 8. Each of these hybrids was made by crossing two different inbred parents, one highly resistant, having an index of resistance of approximately 70, and the other having an index of resistance varying from 0 to 69. These hybrids were divided arbitrarily into four groups according to the resistance of the less resistant of the two parents. Table 8 shows no great differences in resistance among the four groups of hybrids, and it would seem that the resistance of the hybrids was mainly determined by the more resistant of the two parents. Thus the resistance of the parents, with the possible exception noted in the following paragraph, does not have an apparent significant cumulative effect in most hybrid combinations.

TABLE 8.—*Influence of the two parents having different degrees of resistance upon the resistance of the hybrids to bacterial wilt; 1935 trials*

Groups of hybrids, the more resistant parent of which had an index of resistance of approximately 70, and the less resistant parent, an index of resistance varying from 0 to 69	Hybrids	Maximum height of hybrids and parents	Average silking date of hybrids and parents	Average index of resistance of hybrids and parents
	Number	*Inches*		
1, index of resistance of less resistant parent from 0 to 20:				
Hybrid	8	78	Aug. 3	60
More resistant parent		66	Aug. 5	70
Less resistant parent		63	July 31	14
2, index of resistance of less resistant parent from 21 to 40:				
Hybrid	16	75	Aug. 6	65
More resistant parent		64	...do...	69
Less resistant parent		58	Aug. 5	34
3, index of resistance of less resistant parent from 41 to 60:				
Hybrid	14	77	Aug. 6	64
More resistant parent		68	...do...	68
Less resistant parent		59	..ug. 5	49
4, index of resistance of less resistant parent from 61 to 69:				
Hybrid	6	79	Aug. 5	66
More resistant parent		64	Aug. 6	69
Less resistant parent		70	Aug. 4	68

DEGREES OF PARENTS' RESISTANCE IN RELATION TO DOMINANCE

In the results of 1934 there were some indications that when a hybrid was made of inbreds one or both of which had a relatively high resistance, the resistance of the hybrid was usually about as high as that of the more resistant parent. On the other hand, when a hybrid was made of inbreds both of which had a relatively low resistance, the resistance of the hybrid was usually higher than that of either parent. These occurrences were particularly apparent in the trials of 1935, the results of which are presented in table 9. Thus in the class of tall-late hybrids for instance, in group A, the indices of resistance for the two parents are 39 and 24, and that for the hybrid is 50. The index of resistance of the hybrid exceeds that of the more resistant parent by 11 points. On the other hand, in group B, the indices of resistance for the two parents are 68 and 46, while that for the hybrid is 65, a value closely approximating that of the more resistant parent. Similar relationships are found in the other three classes of hybrids in table 9. So far the explanation of these relationships is not clear. However, a working hypothesis partly concerned with hybrid vigor is offered under Discussion.

IMPORTANT EXCEPTIONS TO GENERAL RULES REGARDING HYBRID RESISTANCE

Some exceptions to the already stated general rules on hybrid resistance have been found in almost all experiments. Some of these exceptions may have been due to variations in the material or environment, others to errors in technique, while still others, and particularly some of the more striking ones, may be inherent in the problem. For instance, there were a few hybrids which showed a low degree of resistance in spite of the fact that one of the parent strains had a high index of resistance. Some other hybrids showed resistance intermediate in degree between that of the parents. Such exceptional cases would require special study and interpretation. These cases

seem to suggest that the mode of transmission of the resistance qualities may be of more than one type and that it may depend upon the material employed.

TABLE 9.—*Influence of parents' resistance upon resistance of hybrids to bacterial wilt in cases of hybrids produced from low-resistant and high-resistant parents; 1935 trials*

Kind of hybrid[1]	Hybrids	Maximum height of hybrids and parents	Average silking date of hybrids and parents	Index of resistance of hybrids and parents
	Number	*Inches*		
Tall-late:	29			
A, index of resistance of neither parent exceeding 50:				
Hybrid	13	77	Aug. 7	50
More resistant parent		62	Aug. 6	39
Less resistant parent		57	Aug. 5	41
B, Index of resistance of one or both parents exceeding 50:				
Hybrid	16	82	Aug. 7	65
More resistant parent		64	...do.....	68
Less resistant parent		61	Aug. 6	46
Average:				
Hybrid		80	Aug. 7	58
More resistant parent		64	Aug. 6	55
Less resistant parent		59	Aug. 5	39
Short-late:	31			
A, index of resistance of neither parent exceeding 50:				
Hybrid	20	69	Aug. 8	56
More resistant parent		62	Aug. 6	36
Less resistant parent		57	Aug. 5	28
B, Index of resistance of one or both parents exceeding 50:				
Hybrid	11	67	Aug. 7	63
More resistant parent		60	Aug. 5	66
Less resistant parent		61	...do	41
Average:				
Hybrid		68	Aug. 8	59
More resistant parent		61	Aug. 5	47
Less resistant parent		59	...do	33
Tall-early:	40			
A, index of resistance of neither parent exceeding 50:				
Hybrid	13	77	Aug. 3	60
More resistant parent		60	Aug. 6	40
Less resistant parent		56	Aug. 5	29
B, index of resistance of one or both parents exceeding 50:				
Hybrid	27	77	Aug. 3	66
More resistant parent		63	Aug. 4	67
Less resistant parent		57	Aug. 3	37
Average:				
Hybrid		77	...do.....	64
More resistant parent		62	Aug. 4	58
Less resistant parent		56	Aug. 3	32
Short-early:	50			
A, index of resistance of neither parent exceeding 50:				
Hybrid	26	69	.. do.....	50
More resistant parent		60	Aug. 4	42
Less resistant parent		58	Aug. 3	25
B, index of resistance of one or both parents exceeding 50:				
Hybrid	24	70	Aug. 2	67
More resistant parent		60	...do.....	66
Less resistant parent		59	Aug. 3	40
Average:				
Hybrid		69	Aug. 2	58
More resistant parent		60	Aug. 3	54
Less resistant parent		59	...do.....	32

[1] See footnote 1, table 2.

Some early resistant hybrids have been obtained by taking advantage of the fact that in crossing two inbreds, the resulting hybrid in some cases was earlier than either of the two parents. Such differ-

ences in maturity between inbred parents and hybrids ordinarily varied from 1 to a few days, and in a few exceptional cases, to 2 weeks.[11] These relatively earlier hybrids in most cases did not show a lower degree of resistance than either of their later maturing parents. However, in the very few exceptional cases where the difference in maturity between parents and hybrids was relatively great, from 10 to 14 days, the hybrids did show an appreciably lower degree of resistance than that of either parent.

Early resistant hybrids were also produced by crossing an early resistant inbred possessing "true resistance", with another inbred of the same type, or with another early inbred regardless of its degree of resistance. These hybrids have so far withstood the severest inoculations without appreciable reduction in yield at the time of harvesting for canning. Like all the rest of the hybrids and inbreds inoculated, they showed the characteristic leaf stripes, but appeared to recover from the disease as growth progressed.

DISCUSSION

In order to clarify some of the more pertinent concepts in this paper, a brief discussion follows of (1) the resistance of the inbred lines, (2) the height and lateness of the hybrids in regard to resistance, and (3) the inheritance of resistance.

The inbred lines may for convenience be classified in four categories on the basis of resistance: (1) Those with resistance correlated mainly with height, (2) those with resistance associated chiefly with lateness, (3) highly resistant lines with resistance not correlated with either of these characters, and (4) those with a very low degree of resistance, even though they are relatively tall and late. The last two categories of inbreds are very rare, probably comprising less than 0.5 percent of all the inbreds tested. The majority of the inbred lines, however, possess resistance which is associated with height and lateness of the plants. In most cases it would be difficult to determine to what exact degree the resistance of a particular inbred is associated with its height and lateness.

In the hybrids as in the inbreds, height and lateness seem to be associated with resistance. Relatively late hybrids as a rule showed greater resistance than relatively early hybrids. The data in tables 2 and 4 for a group of 36 hybrids with one common parent consistently show that the tall-late hybrids have a higher index of resistance than the short-early hybrids. The same association is even more strikingly brought out in the data for 229 hybrids, shown in table 5, where the tall-late hybrids have an index of resistance of 53 while the short-early hybrids have an index of 29, a difference of 24 points. The difference in height between these two groups was only 10 inches, and the difference in date of harvesting was only 6 days. Individual hybrids differing in height by about 2 feet and in silking date by more than 14 days showed differences in resistance of 80 or more points.

The correlation between height and lateness and hybrid resistance seems to diminish from year to year as the "true resistance" of the composing inbreds increases. Thus in 1933 the difference in resist-

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[11] These differences in maturity between the inbreds and the hybrids seemed to vary to a certain degree with the season. In 1934 they were more common than during the other two seasons. In the majority of cases, however, the hybrids did not appreciably differ in maturity from the parent inbreds.

ance between the tall-late and short-early hybrids was considerable
and the most resistant hybrids were found only among the tall-late
class of hybrids.   In 1934, because of the number of early, relatively
resistant inbreds employed in their making, the hybrids showed less
tendency to correlate resistance with height and lateness, as many
of the early hybrids showed considerable resistance.   Still, the
hybrids tested in that year as a whole showed a definite correlation
between height and lateness on the one hand, and resistance on the
other, as may be seen in table 5.   The hybrids tested in 1935 were
derived mainly from relatively early-resistant inbreds and as a whole
showed hardly any correlation between height, lateness, and resist-
ance (table 9).   Nevertheless, some of the most resistant hybrids even
in that year were among the tall-late class, and some of the most sus-
ceptible ones, among the short-early class.

The inheritance of resistance from the inbreds to the hybrids seems
evident from data presented in tables 3, 5, 7, 8, and particularly
table 9.   That the low-resistant inbreds (groups A of table 9) pro-
duced as a rule less resistant hybrids than the high-resistant inbreds
(groups B) is apparent from the index of resistance of these hybrids
in each of the four classes shown.   These indices are 50 and 65, 56
and 63, 60 and 66, 50 and 67.   In general, these results confirm those
obtained in 1934 (table 5), although some exceptions have been noted.

The dominant character of resistance is evident in these results.
In each group or class of hybrids shown in table 9, the index of resist-
ance seems to approach that of the more resistant of the two parents,
and in the case of the A groups it seems to exceed that of the more
resistant parent.   This increase in resistance of some hybrids, of those
produced from low-resistant inbreds, over the resistance of the inbred
parents may be attributable to the additive effect of a great number
of resistance factors.   From the evidence available, however, it seems
more likely that it is associated with the increased height resulting
from hybridization, as has already been observed in the case of the
1933 and 1934 hybrids as a whole.

An explanation as to why hybrid vigor may be associated with
considerably increased resistance in the case of hybrids produced from
low-resistant inbreds, and may not be so associated in the case of
hybrids produced from high-resistant inbreds, seems desirable.   To
obtain a satisfactory explanation would require the performance of
additional special tests.   As a working hypothesis, however, it may
be assumed (1) that each of the three types of resistance, height-
correlated, lateness-correlated, and "true resistance", within a hybrid
has a certain value, and (2) that the resistance of the average hybrid
is not ordinarily expressed by the sum total of the values of the three
resistance types but rather by the highest one of the three values;
i. e., the three types of resistance have only a minor, if any, additive
effect, and the highest prevails over the other two.   If such an
assumption is correct, then, in the case of average hybrids produced
from low-resistant inbreds, the height-correlated resistance of the
hybrids might exceed the resistance value of the inbreds.   Thus, the
hybrid would have an index of resistance higher than that of either of
the two parent strains.   However, in the case of hybrids produced
from high-resistant inbreds (in many cases the inbred's resistance
may be largely of the "true resistance" type), the transmitted resist-
ance of the parents might exceed the height-correlated resistance of

the hybrids. In such cases the index of resistance of the hybrids would be approximately equal to that of the more resistant parent strain.

The data have shown that as a whole when hybrids are made from highly resistant inbred strains, the resistance of these hybrids is governed primarily by the resistance of the inbreds. In such cases the height and the lateness of the resulting hybrids seem to play a secondary role. It is from such early resistant inbred lines, obtainable by careful searching, inbreeding, selecting, and continuous testing by artificial inoculation, that tall and vigorous, early resistant hybrids may be produced to serve the needs of the canners and market gardeners.

SUMMARY

Studies on resistance of inbred strains and crosses of sweet corn to bacterial wilt of maize caused by *Phytomonas stewarti* were made during the growing seasons of 1933, 1934, and 1935. The sweet corn tested for resistance included approximately 1,000 inbred strains and 1,000 F_1 hybrids and top crosses of the Golden Bantam type. The plants were tested by artificial puncture inoculation and by natural infection, when that occurred. Preliminary trials were made to determine comparative efficiency of various methods of inoculation, period of greatest host susceptibility, age of host plant in relation to type of symptom expression, comparative rate of wilting of hybrids and their respective inbred parents, time for final record taking, uniformity of inoculation, and comparative value of artificial puncture inoculation and natural infection. Artificial inoculation was found to have certain important advantages over natural infection as a method of testing for resistance. The results obtained are as follows:

Great differences in resistance were found among the inbred strains. As a rule, the taller strains showed greater resistance than the shorter strains, and the later strains showed greater resistance than the earlier strains. A few relatively short-early strains showed a high degree of resistance, and a few tall-late strains showed a low degree of resistance.

The hybrids and the top crosses, like the inbreds, showed wide variations in resistance. The resistance of the hybrids was found to be inherited from the inbred parents. Resistant inbreds in most cases produced resistant hybrids, and susceptible inbreds produced susceptible hybrids.

Resistance in the hybrids appeared to be generally dominant. As a rule, crosses between resistant and susceptible inbreds gave resistant hybrids, crosses between resistant inbreds and resistant inbreds gave resistant hybrids, and crosses between susceptible inbreds and susceptible inbreds gave susceptible hybrids.

Tall and late hybrids were found to be, as a rule, more resistant than short and early hybrids.

Hybrids produced from highly resistant inbreds generally showed high resistance regardless of their degree of earliness or lateness. Highly resistant hybrids were commonly late although a few were early.

Hybrid height usually indicating hybrid vigor as a rule increased resistance, particularly in the case of hybrids produced from relatively low-resistance inbreds.

The top crosses reacted like the true hybrids in all respects.

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